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ABSTRACT

These proceedings of a symposium designed to increase public awareness of current research in biology and biotechnology include: welcoming addresses by Chau Sian Eng and S. Radhakrishna; an opening address by Tay Eng Soon; five papers; four abstracts; summary; symposium program; and list of participants. The five papers are: (1) "The Role of Biotechnology in the Economy of Developing Countries" (John Smith); (2) "Gene Transfer and Somaclonal Variation for Plant Improvement" (R. D. Brock); (3) "Recent Advances in Biotechnology with Special Reference to Developing Countries" (K. F. Gregory); (4) "The Application of Recombinant DNA Techniques for the Study of Human Genetic Diseases" (J. Tam, K. Tan-Un, Y. Woo, and V. Lam); and (5) "Brem Bali Fermentation" (Indrawati Gandjar, Dewi Slamet, and Isworo Rukmik). The abstracts are "Current Research in Biotechnology in India" (H. Sharat Chandra); "Biotechnology of Interferons: The Transition from Natural to Recombinant Human Interferon" (Y. H. Tan); "Recombinant DNA Derived Human Interferon" (Budd Colby); and "New Sources of Single Pore Cell Protein" (Yasuju Minoda). The symposium demonstrated clearly that biotechnology encompasses a wide variety of techniques and applications; moreover, because developing countries are widely diverse, the role and application of biotechnology cannot be generalized across all nations. (JN)

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PROCEEDINGS OF THE SYMPOSIUM ON RESEARCH IN BIOLOGY AND BIOTECHNOLOGY IN DEVELOPING COUNTRIES

NOVEMBER 2 — 4, 1983

(SELECTED PAPERS)

ASIAN NETWORK FOR BIOLOGICAL SCIENCES

BOTANY DEPARTMENT N.U.S.

ZOOLOGY DEPARTMENT N.U.S.

SINGAPORE SCIENCE CENTRE

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PROCEEDINGS OF THE SYMPOSIUM ON BIOLOGY AND BIOTECHNOLOGY IN DEVELOPING COUNTRIES

Edited by A N Rao

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National University of Singapore, November 2–4, 1983

Edited by:

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Lower Kent Ridge Road, Singapore 0511

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SYMPOSIUM ON
RESEARCH IN BIOLOGY AND BIOTECHNOLOGY
IN DEVELOPING COUNTRIES
2 - 4 NOVEMBER, 1983

Dr Tay Eng Soon, Minister of State for Education, addressing the gathering.

SYMPOSIUM ON
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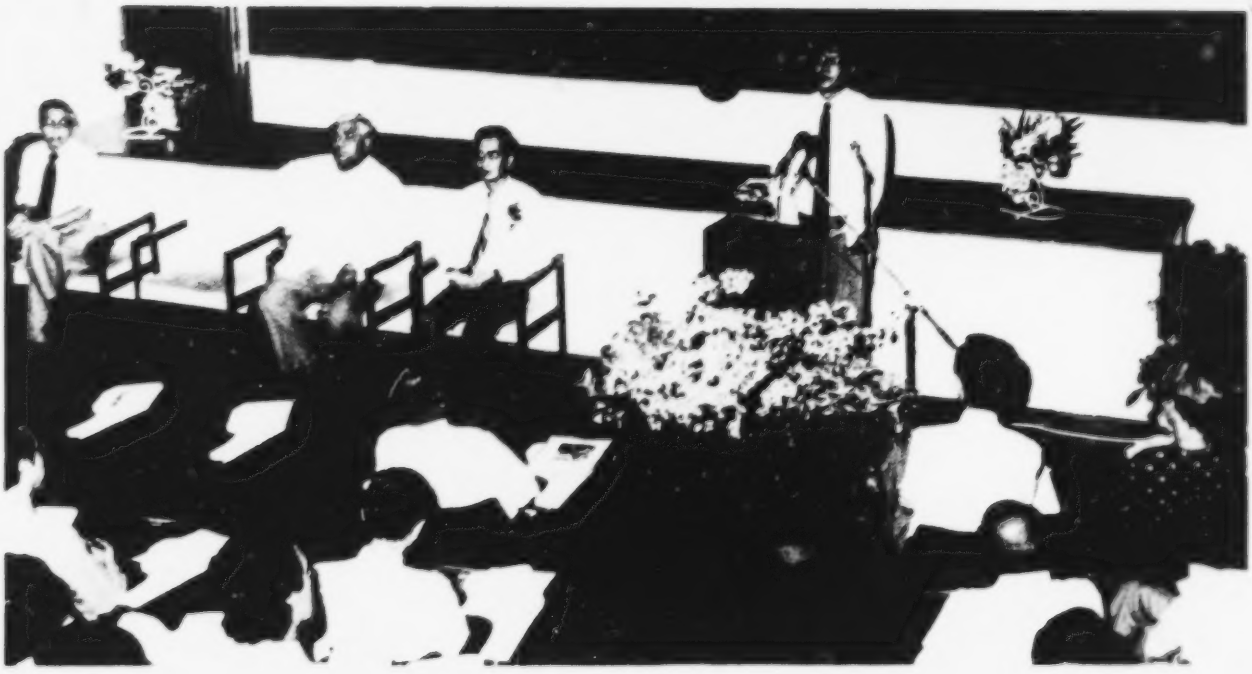

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Welcome Address

Chua Sian Eng

President, Singapore Institute of Biology
Ministry of National Development, Maxwell Road, Singapore 0106

It is a great pleasure for me to welcome our distinguished guests and participants to this Symposium on Research in Biology and Biotechnology in Developing Countries. The purpose of this Symposium is for all of us to get to know one another and to exchange ideas in this interesting field of biotechnology. We have among us distinguished participants and scientists of international repute who are specialists in biotechnology.

Scientists in the field of biology have always been intrigued by the principles of heredity and variation but until recently have made very few inroads into altering the genetic material itself. However, this situation is rapidly changing. After just a few years of experimentation, the recombinant DNA revolution is gearing up, through the introduction of high precision technologies, for directly manipulating the genetic material of any organism, be it virus, bacterium, plant or animal.

In a paper by Prof. K F Gregory, Chairman of Department of Microbiology, University of Guelph, Canada, biotechnology can be defined as the "utilisation of a biological process, be it microbial, plant or animal cells, or their constituents, to provide goods or services". According to him, biotechnology is not new for the production of ethyl alcohol and lactic acid from foods predates written history. During World War I, acetone for ammunitions was produced by bacteria in Britain, Canada and United States, while glycerol for ammunitions was produced by yeast in Germany. Subsequently, the fermentation industry received a big impetus from the discovery of penicillin and other therapeutically important antibiotics. Various enzymes, amino acids and an increasing number of other products began to be produced by micro-organisms on a commercial basis. Biotechnology developed over the past few decades, is almost unnoticed by the average person. Suddenly in the eighties, it has emerged with a considerable amount of publicity. Products produced on a commercial scale by micro-organisms are some 91 antibiotics, 10 organic acids, 2 solvents, 9 vitamins, 21 amino acids and an assortment of other compounds. Some of the products of biotechnology produced in very large amounts are critic acid (about 100 million kilograms per year) which is used in many consumer products such as soft drinks, monosodium glutamate (about 200 million kilograms) which is a flavour enhancer. Japan's Ministry of International Trade and Industry recently reported that the products of biotechnology in that country were valued at 18.2 billion dollars per year and predicted that this would rise to 29.5 billion dollars by the year 2000.

It is not possible for our Symposium to include all aspects of biotechnology. However, we will endeavour to highlight the relevant researches that are currently being carried out by the participants present here today. The participants will discuss the usefulness and application of biotechnology in the field of agriculture, biology and biochemistry. I hope that this Symposium will serve to raise the level of public awareness of researches that are being carried out in Singapore and in other countries.

It leaves for me now to wish all of you a fruitful participation.

Welcome Address

S Radhakrishna

Scientific Secretary
Committee on Science & Technology in Developing Countries (COSTED)
Department of Physics, Indian Institute of Technology, Madras 600036, India

On behalf of COSTED, Committee on Science and Technology in Developing Countries, I have great pleasure in welcoming you to participate in the Symposium on Research in Biology and Biotechnology in Developing Countries. We are very happy that we have been able to organise this very important and topical conference in co-operation with the Singapore Institute of Biology and the National University of Singapore. This is one among a series of important seminars organised by COSTED on emerging areas. Biotechnology is a very important area for developing countries. Unlike other high technologies it is not expensive, is not energy intensive and does not require costly equipment. Any conference organised by the Botany Department in your university seems to attract considerable international attention. The earlier conference on Tissue Culture of Economically Important Plants and the resulting publication received international reviews and COSTED felt very happy to entrust another major conference to this department. We are indeed very gratified to note that this conference has attracted over 150 original papers thereby indicating the extent of scientific work going on in this important area. I would like to compliment the Asian Network for Biological Sciences under the leadership of Prof. Hiroshi Harada and Prof. A N Rao Chairman and Secretary of ANBS respectively, for undertaking impressive list of activities including seminar workshops and training programmes.

The COSTED has been organising several activities in developing countries. In particular, these activities include:

(a) Formation and promotion of regional organisations where scientists from developing countries plan and implement programmes of use and interest to them.

(b) Preparation of text books, teaching aids, laboratory equipment and support material for meeting the needs of institutions in developing countries.

(c) Organise major international seminars on emerging areas such as this in developing countries.

and (d) Organise training workshops for scientists from developing countries.

It is our policy to promote and foster the exchange of information among the scientists of different disciplines, especially those in developing countries. The present symposium is supported with this objective.

I wish all of you a pleasant stay in Singapore and successful deliberations.

OPENING ADDRESS

Dr Tay Eng Soon
Minister of State for Education
Singapore

I have much pleasure in being present at the opening of your Symposium on Research in Biology and Biotechnology in Developing Countries. I understand that this symposium has attracted participants from over 16 countries. May I take this opportunity to welcome you most warmly to Singapore. From the information which I received on the Symposium, I note that there will be more than 140 papers presented at the conference, all within a short space of only 3 days! I hope that this very tight schedule will not prevent you from seeing something of Singapore before you leave. Many experts today seem to be agreed that the major technological impetus for the rest of this century will come from two main fields. These are, firstly, the micro-electronic revolution and associated with it the computer. And secondly, the biotechnology revolution.

The first is already making a tremendous impact on the way we communicate at long distances and the way we can handle vast quantities of data. The information technology that we have today and the automation that can be achieved through computers are changing our life styles. For example, industrial robots are taking over unpleasant and dangerous occupations in the factory. Microcomputers and word processors are improving productivity in the banks and offices. Distances have shrunk as telecommunications become cheaper and more widely available. There is even the promise that by the end of this decade super fast computers will become available, and with them, systems which will be able to imitate human intelligence itself. I am told these intelligent systems would be able to do what can be done only by human beings today e.g. in medical diagnosis or in simultaneous translations of speech.

The second field is a subject which will be discussed in your conference. In some senses, biotechnology is much older than micro-electronics. It started when man discovered how to turn fruit juices into wine by fermentation and how to make dough rise by using yeast. But today we are at the threshold of a whole new field of possibilities as new discoveries are being made on how to manipulate the gene. These discoveries could have profound impact on some of the major problems which face mankind.

In food production, any biotechnological solutions to the problems of plant disease or to the way plants can fix their own nitrogen could radically change the problem of global food shortage. Similarly, in the medical field, some of the most difficult problems such as cancer may eventually yield to drugs developed by means of genetic engineering. How can developing countries exploit these new technologies to their own advantage? The answer to this question is complex. Much would depend on the size of the country, its economy, how well developed and diversified it is and so on. Fortunately, nowadays, there are relatively few barriers to the free flow of information and expertise. For example, the discovery of new strains of high yield rice at the International Rice Research Institute in the Philippines become available to other rice growing countries within a short time. Similarly, new formulations of pesticides or fertilizers soon become available in the open market. The problems at the local level are those associated with disseminating the new knowledge or techniques. It has to do with educating the end-user, to overcome ignorance and resistance to change. I have deliberately not brought in the complex sociopolitical problems of land ownership, and the control of markets and licences which exist in many countries. That is a subject for another forum.

But on the question of assimilating the new technologies and disseminating them, the crucial group of people involved are those who have been able to master these technologies to the point when they can adopt and apply them to local problems or local industries. In the case of biotechnology, they would be the research scientists and biologists who are sufficiently knowledgeable about biotechnology applications on the one hand and are familiar with local problems on the other. They are the essential agents for technology transfer and assimilation.

In the Singapore context, our largest group of biologists are at the National University of Singapore. I understand that a number of them have begun research work in the biotechnical field. However, at present the effort is isolated and fragmented. To achieve more, it may be necessary for the relevant departments at the University to come together to form a multi-disciplinary unit or team which can focus its effort on this important area. Fortunately, funding is or should be no problem as the Government has set aside $50 million for supporting research activities. Similarly, staffing quotas have also become generous enough for some staff to devote their full time to research work. All the conditions therefore favour a significant increase in research at the University.

Research in biotechnology or genetic engineering at the University may not lead to the major breakthroughs that occur in better endowed centres elsewhere. But the real value is that it could serve as the breeding ground for a new generation of biotechnologists who will be needed in our industries a decade from now. A decade ago, the Engineering Faculty at the University foresaw the need to train their engineering students in the new technologies of digital electronics, microprocessors and automation. Their efforts have paid off because today, many of their graduates are actively involved in the new high technology companies which are operating in Singapore.

I believe that the life sciences departments in the University should have a similar vision with respect to biotechnology and the impact it will have in our economy in the future. In closing may I wish all the participants a pleasant and stimulating time.

I have pleasure now in declaring the Symposium on Biology and Biotechnology open.

The Role of Biotechnology in the Economy of Developing Countries

John E Smith

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WHAT IS BIOTECHNOLOGY

Biotechnology is the science of applied biological processes and involves the practical application of biological organisms, systems or processes to manufacturing and service industries and to environmental management. Whereas bioscience involves acquisition of biological knowledge biotechnology is primarily concerned with the application of biological knowledge. In practice biotechnology will utilise bacteria, yeast, fungi, algae, plant cells and animal cells as essential components of industrial processes. To achieve the optimum application of biotechnology will require the integration of several disciplines including microbiology, biochemistry, molecular biology and chemical and process engineering.

Although biotechnology will have as its primary aim the furtherance of industrial production it will also be of value to the management of environmental problems, including waste treatment, pollution control and integration with non–biological technologies. Environmental biotechnology is becoming increasingly recognised as a major growth aspect of biotechnology in Western Nations not only because of pollution control but also because of the ability to produce high and low value products from otherwise waste non-utilisable materials.

On the industrial scale biotechnology involves fermentation processes (including beers and wines, bread, cheese, antibiotics and vaccines), water and waste treatment, aspects of food technology and an increasing range of novel applications ranging from biomedical to metal recovery from low grade ores. Although it is not possible to accurately predict future world market trends, recent predictions confidently forecast that by the year 2000 world wide markets (in $ US billions) should approximate to: energy, 16.4; foods, 12.7; health care, 9.1; chemicals, 10.6; agriculture, 8.6; and others, 7.7

There can also be no doubt that in the long term biotechnology offers a means of solving some of the world's pressing problems in particular those related to medicine food production, pollution control and the development of new energy sources. The governments of virtually all western nations are pushing biotechnology politically and financially and in most cases have produced impressive reports about the benefits of biotechnology, the necessary future developments and the main areas which need to be stimulated and developed. The picture in developing countries is less clear and it will be one of the main hopes of this Conference to stimulate national awareness of the benefits of biotechnology and to see how the correct areas of biotechnology can be developed in individual developing countries. There is not one simple solution or simple application and each nation must chart its own course in the application of biotechnology.

THE EVOLUTION OF BIOTECHNOLOGY

In basic practice biotechnology is not a new subject but rather has its origins far back in antiquity and almost every country has historical involvement with biotechnology. Four major developmental phases can be identified in achieving the fullness of modern biotechnological practices.

1. Food and Beverage Biotechnology

Processes such as brewing and wine making and leavening of bread have been practised for several thousand years; the ancient Sumarians and Babylonians were drinking beer by 6000 BC, the Egyptians were baking leavened bread by 4000 BC, and wine has been in accepted practice in the Near East since at least the time of the book of Genesis. That these processes were being carried out by microorganisms namely the yeast **Saccharomyces** was not to be recognised until the 17th century with the development of the light microscope.

Other ancient microbiologically based processes include fermented milk products, cheeses and yoghurts, and many types of oriental foods, soya sauce, tempeh. Similarly, the practice of growing large mushrooms for food, such as **Agaricus bisporus, Lentinus edodes** and **Volvariella volvaceae** are of variable antiquity and are now cultivated throughout the world.

Although it is possible to carry out all of these processes with relatively simple or low technology modern commercial practice requires a high element of sophisticated technology. Furthermore, many of these biotechnological products represent significant financial rewards not only to industry but also to national economies. The economic returns from these old established processes dwarf those of the exciting products from new technology (Tables 1 and 2).

Table 1. Fermentation Products: According to Industrial Sectors

Sector	Activities
Chemicals:	
organics (bulk)	ethanol, acetone, butanol, organic acids (citric, itaconic)
organic (fine)	enzymes perfumeries polymers (mainly polysaccharides)
inorganic	metal beneficiation, bioaccumulation and leaching (Cu, U)
Pharmaceuticals	antibiotics diagnostic agents (enzyme, monoclonal antibodies) enzyme inhibitors steroids vaccines
Energy	ethanol (gasohol) methane (biogas) biomass
Food	dairy products (cheese, yoghurts), fish and meat products beverages (alcoholic, tea and coffee) baker's yeast food additives (antioxidants, colours, flavours, stabilizers) novel foods (soya sauce, tempeh, miso) mushroom products amino acids, vitamins starch products glucose and high fructose syrups functional modifications of proteins, pectins
Agriculture	animal feedstuffs (SCP) veterinary vaccines ensilage and composting processes microbial pesticides Rhizobium and other N–fixing bacterial inoculants mycorrhizal inoculents plant cell and tissue culture (vegetative propagation, embryo production, genetic improvement)

Adapted from (Bull, Holt and Lilly, 1982)

2. Products initially developed from microbiological processes under non-sterile conditions

By the end of the 19th century microbial fermentations were producing large commercial volumes of ethanol, acetic acid, organic acids, butanol, acetone etc. The fermentation systems were simple and achieved selective microbial control by careful manipulation of the environment without the involvement of complex mechanical systems. With the development of the petrochemical industry it became possible to produce these chemicals more cheaply than microbial fermentation and in most cases their production decreased substantially. However, in recent times paralleling the escalation in oil prices there has been a worldwide resurgence in fermentation practice.

Yet other outstanding example of this approach has been processes associated with waste water treatment and municipal composting of solid wastes. In particular the decomposing and detoxification of human sewage has been invaluable for reducing the incidence of many types of disease. Biotechnological treatment of waste waters represents by far the largest fermentation capacity practised throughout the world. In many developing countries the achievement of pure drinking water and adequate and safe treatment of human wastes have yet to be realised. Solving these problems would have major benefits to populations removing a large element of the miseries associated with diseases of the alimentary tract such as dysentery.

3. Sterile practice in biotechnological processes

The pathway of biotechnology dramatically changed in the 1940s with the introduction of complicated engineering practices which allowed the mass cultivation of microorganisms in such a way that pure cultures could be grown in fermenters. Thus by prior sterilisation of the medium and the fermenters and with engineering provision for the exclusion of incoming contaminants only the chosen biocatalyst, viz. microbe, enzyme or plant or animal cell, could be in the fermenter. Products produced in this way now represent an increasing volume of modern commercial biotechnology and include antibiotics, enzymes, amino acids, polysaccharides, vaccines etc. Most of these processes are complicated and expensive and suitable only for high value products (Table 2).

Table 2. Total UK Fermentation Capacity

Product	Total Capacity (cubic metres)
Waste Water	2,800,000
Beer	128,000
Baker's Yeast	19,000
Antibiotics	10,000
Cheese	3,000
Bread	700

(from Dunnill, P., 1981)

4. New dimensions for biotechnology

What are the principal scientific breakthroughs which have created the "new biotechnology."

i. Fundamental discoveries in the life sciences particularly the role of DNA as the molecular carrier of genetic information.

ii. Techniques for the manipulation, alteration and synthesis of genetic material (either directly or via cell fusion) to create new life forms.

iii. Techniques based on microbiology for cultivation, screening and selection of useful cells or microorganisms and manipulation of their behaviour under controlled conditions.

iv. Immobilisation of enzymes and whole cells for biocatalytic purposes.

v. Techniques for plant cell and tissue culture for accelerated propagation of useful plants.

vi. Downstream processing techniques for extraction, treatment, purification and conversion of useful materials following biomass production stage.

The rapid advances in new biotechnology threaten to increase rather than reduce the scientific and technical gap between the developing nations and the industrialised countries. The current rapid advances in new biotechnology are presently possible in industrialised countries largely because there exists a large highly skilled workforce. Developing nations will find increasing difficulties in attempting to enter into these areas of activity because in most cases the necessary trained manpower does not yet exist in sufficient numbers to have any major impact.

In practice, biotechnology can be considered as a multifacet subject with an interwoven network of activities which reflect the complex interaction of the component subjects and the areas of application in industry. Biotechnology will employ techniques derived from chemistry, microbiology, genetics, biochemistry, chemical engineering and computer science and the main mission of the practitioners will be the innovation, development and optimal operation of processes in which biochemical catalysis has a fundamental and irreplaceable role. Biotechnology is not really a new discipline but rather the interaction between various parts of biology and engineering. An essential component is the ability of scientists and engineers to understand the language of the other.

Bioscientists and engineers of various specialisations will make their individual contributions to biotechnological processes. The term biotechnologist could be considered as an all-embracing description of bioscientists and chemical engineers engaged in applying their skills and knowledge to the processing of biological materials. A biochemical engineer is a process engineer whose role is to transfer the knowledge of the biological scientist into a practical operation.

5. Selection of biotechnological processes

A centrally important aspect of biotechnological development will be the availability of suitable raw materials and substrates for processing. Substrate costs can amount to between 30 to 70% of the final product cost. Availability of substrates will be dependent not only on technical and economic grounds but also by political consideration. Thus gasohol production from various organic substrates may not offer outstanding economic attraction but politically can present some bargaining power against escalating oil imports.

Biotechnology, in particular, offers major advantages for the conversion of agricultural by-products and other organic wastes to more valuable products. Thus renewable resources from agriculture, forestry and industrial organic wastes are gaining increasing importance since they offer, by biotechnological processing, a politically and strategically important base for food, feed, basic chemicals and energy production. However, to achieve this will require wide ranging programmes not only in the component aspects of biotechnology but also in land management.

Each biotechnological scenario will require continuous assessment of the resources available, the economics and the impact on the environment, together with the health and safety of operators and users. Biotechnology, if correctly planned, should help to achieve a proper balance between natural resources, human needs and the environment.

Furthermore, when considering the applications of biological discoveries to future industrial applications the importance of time should not be underestimated. Between the moment when a new discovery has been made and successful commercial production achieved, 5 to 20 years could have passed.

Biotechnological processes can be examined on the basis of volume and value or on a technological level. The choice will ultimately rely on resource availability and the skill of the available manpower.

(a) Volume and Value

(i) High volume but low value biotechnological processes include the production of methane, ethanol, biomass and animal feed, water purification, and effluent and waste treatment. These systems currently exist in most developing nations and it is here that the first main improvements in the system should be made making use of all available new information. For example, there have been outstanding new developments in biogas producing processes that could be applied to many of the existing processes widely used in developing countries.

(ii) High volume intermediate value products such as amino acids and organic acids, food products, acetone, butanol and baker's yeast. Again many of these products are already being manufacured but the methods of production could be improved.

(iii) Low volume high value products include many health care products such as antibiotics, interferons, enzymes, vitamins etc. It is doubtful whether these products will be readily manufactured in most developing countries.

(b) Technology Level

(i) High level processes such as health care products, human food and food additives involve high capital investment, sophisticated plant, high maintenance costs, high operator skills.

(ii) Intermediate levels involving fermented foods and beverages, animal feeds, pesticides, crude enzymes will involve moderate capital investment and less complex operations.

(iii) Low level operations include naturally adapted mixed fermentations, biogas, mushroom production, traditional fermented foods and beverages and will require small capital investment and scale of operation, simple equipment, labour intensive operations, village level technology.

There are several distinct areas where biotechnology will have major impacts in developing economies:

A. Food and Agriculture

A basic feature common to all developing countries is the need to establish good agricultural practice on which an adequate food supply can be derived. Without sufficient food economies cannot develop. Developing countires play a major role in world economies as the principal producers of coffee, cocoa, and many tropical fruits. Also of importance will be vegetable fats and oils, soya and maniac. Better agricultural management will lead to improved production. This will involve systematic long–term approaches to stimulate self–sufficiency. Many nations were formerly net exporters of food crops but now through population increases and also political instability many have now become net importers of many essential commodities. Although Food Aid programmes may serve a short term need they present long term disadvantages. Productivity in many areas has decreased because of inadequate preservation of soil quality, and by overstocking leading to pasture deterioration. Shortage of animal feed is a widespread problem in many arid areas of developing nations.

There are many agricultural problems that must be realised and appropriate measures taken to improve productivity. How can biotechnology be used to improve agriculture?

(i) **Food crops and Forestry:** Of particular significance will be the area of plant cell biotechnology. Horticultural practice has long made use of vegetative cloning of plant parts (shoots, leaves, etc) for selective and rapid propagation of many plant species. Subsequently, plant tissues as root tips, cambium or plant tumours were found to be relatively easy to grow aseptically in artificial cultures. With careful selection of medium and other environmental factors it was possible with many species to achieve differentiation and eventual regeneration of whole plants. The four stages of the complete cycle are "

(1) establishment in culture;

(2) organogenesis;

(3) plantlet amplification;

(4) establishment in soil.

The major commercial use of this type of tissue culture has been towards the rapid propagation of plants both for stock purposes and for sale.

This ability of the horticulturalist to clone large quantities of identical plants from a single parent in a short period of time highlights the regenerative ability of many plant tissues. This practice is now widely used with many commercial advantages including continuous production throughout the year, rapid production of new varieties obtained by conventional genetics, virus–free stock production from shoot tip cultures and genetic improvements of plants using haploids. Furthermore, this technique could be of immense value for eventual mass production of genetically modified cells derived from protoplast fusion or recombinant DNA technologies. Tissue culture can also be used in the long–term storage of specialised germplasm, providing stable genetic material, reducing storage space and decreasing maintenance costs.

At present protoplast fusion represents one of the main tools in plant cell biotechnology. Protoplasts can be prepared from most plant species but only a limited number can regenerate cell walls, divide and ultimately differentiate into normal plants. Many important crop plants are in the latter category. The production of novel somatic hybrids by fusion of isolated plant protoplasts is recognised as an important technological advancement in the plant sciences.

In practice, following induced fusion, protoplasts in liquid culture regenerate cell walls and undergo mitosis, creating a mixed population consisting of parental cells, homokaryotic fusion products and heterokaryotic fusion products or somatic hybrids. Somatic hybrids can be identified and recovered generally through expression of genetic markers, physiological growth requirements or alternatively by micromanipulation. Protoplast fusion between divergent species can sometimes produce hybrids but these are invariably sterile, precluding integration into a breeding programme. A role for somatic hybrids in crop improvement can only be realised if the hybrids can be integrated into conventional breeding programmes. As yet no new varieties have been established in agricultural practice by protoplast fusion technology. However, this will undoubtedly occur in the near future and open up a new dimension of creative and beneficial agricultural advancement.

(ii) **Animal Products:** Animal production can be greatly improved by ensuring healthy animals. Vaccines for many diseases have now been developed and others will surely follow particularly exploiting genetic engineering principles. Cheaper production techniques will arise through biotechnology allowing wider distribution and availability of products such as vaccines.

(iii) **Species Preservation:** Most of the 50 main food crops in the world have been derived from developing countries and plant breeders must return to seek new genetic factors. Indeed developing countries contain most of the world's species and main centres of genetic diversity' Cryopreservation techniques for plant germplasm must be established.

(iv) **Post–harvest Techniques:** Vast quantities of agricultural products are lost because of biodeterioration by insects and microbes. Biotechnology can help to prevent this. Similarly, processing of products is generally poor and wasteful and could be greatly improved by utilising the information available from downstream processing of biotechnology.

6. Human Health and Welfare

Mortality of infants between 2–4 years in developing countries is 40 X higher than developed countries. The contributing factors include protein calorie malnutrition, parasitic infection and gastrointestinal diseases.

There is a need for basic infrastructure to support public hygiene, preventative medicine and basic nutrition.

Much of the present commercial involvement in biotechnology is associated with pharmaceutical products such as interferons, insulin, monoclonal antibodies. There are invariably high value products requiring sophisticated

technology for their formation. It is doubtful if this level of biotechnology will find ready application in most developing economies. The need for it goes without saying but realism must prevail and it would be foolish for developing economies to place this area of biotechnology as high priority. Such products will largely, for the foreseeable future, be imported when needed. It must be hoped that the new genetic engineering techniques will allow easier and cheaper development of vaccines for the many tropical and subtropical diseases.

Better agriculture and medicine will not be pointless achievements. Many developing nations are already showing major reductions in birth rate, e.g. China – 34%, Cuba – 47%.

7. Environmental Biotechnology

Environmental biotechnology can have major impacts in the creative utilisation of agricultural, human and animal wastes and in the development of pure drinking water.

Products that are obtainable from waste treatment include energy (biogas, ethanol), compost, fertilisers, fodder, organic chemicals, etc.

Many developing countries do not have liquid fossil fuel reserves and this places major constraints on their economies and they are in real danger of falling further behind the expanding economies of the developed nations. Alternative sources of energy can be developed using biomass as the starting material. The Brazilian gasohol programme has lead the way in biotechnological innovation and will be paralleled in some form or other by other countries. Biogas production is a long established energy generating process and even in developed nations this anaerobic fermentation process is being re-examined and shown to be a process of much greater value than had formerly been considered. Methods for using lignocellulosics for energy generation have been attempted using aerobic and anaerobic fermentation processes. The complexity of chemical structures hinders rapid progress and it is distinctly possible that thermochemical processes and gas pyrolysis may well be the most efficient means of using lignocellulosics for energy generation.

THE WAY AHEAD

Biotechnology already exists in developing nations at various levels of activity: brewing, food fermentations, waste treatment, biogas, vaccines, antibiotic production etc. The furtherance of biotechnology can be seen in three ways:

1. Improve the basic and applied knowledge of those technologies already practised.
2. Promoting appropriate, locally relevant solutions and uses in biotechnology – responding to local conditions and needs.
3. Training needs for biotechnology.
   (a) Overseas specialised programmes in Microbiology, Biochemistry, Chemical & Process Engineering, Applied Genetics;
   (b) Short specialist programmes "in house" where many local people can participate;
   (c) Creating strong institutions.

In the final answer each country must seek out the relevant areas of biotechnology that are appropriate for support within their own economies and act appropriately. The time for action is NOW.

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Gene Transfer And Somaclonal Variation For Plant Improvement

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ABSTRACT

Plant improvement comprises three phases of activity. First, generation of variability. Secondly, selection of improved genotypes. Finally, comparative tests to demonstrate the superiority of the selected genotypes. Molecular genetics will contribute to the first two phases by making possible the transfer of individual genes to generate specific variability, thereby greatly improving selection efficiency. Cell culture has already contributed by enabling selection for appropriate characters to be applied to cells rather than to whole plants, thereby greatly reducing the time and cost of the selection phase. The process of taking plant tissue into cell culture and then regenerating plants induces somaclonal variation from which useful agronomic selections have already been made.

Recombinant DNA technology, using restriction endonucleases, permits the cutting and rejoining of DNA molecules and the insertion of specific pieces of DNA into plasmids for multiplication (cloning) and into molecular vectors for transfer to other genotypes (transformation). All of the techniques for gene selection and cloning and for transformation of agricultural plants have not yet been developed. The current status of the gene transfer techniques and of somaclonal variation is reviewed and their potential for plant improvement is discussed.

PLANT IMPROVEMENT

Plant improvement comprises three phases of activity. First, generation of variability. Secondly, selection of improved genotypes. Finally, comparative tests to demonstrate the superiority of the selected genotypes.

Man first used the natural variability that had been accumulated throughout evolution and selected plants useful for food and fibre. Plant breeders still look to the variability conserved in varieties, land races and germplasm collections as their first source of useful genotypes. Sometimes commercially useful varieties are selected directly. More often useful characters are identified, but these are usually associated with undesirable traits. The plant breeder then resorts to hybridization in an attempt to combine the most desirable characters of different parent plants. A range of sophisticated hybridization and selection techniques are now available and these have greatly accelerated the rate of progress in plant improvement. But the random reassortment of genetic traits during sexual reproduction dictates that the occurrence of the desired combination of traits in the progeny remains a very rare event. Progress in plant breeding is very dependent upon population size. It is a 'numbers game', and a costly and time-consuming process. Even now the production of a new wheat variety in North America, Europe or Australia still takes 10 years and costs $1 million.

As plant breeding proceeds and better varieties are developed, further improvement becomes progressively more difficult. It becomes important not only to retain more and more of the characters of the best, high yielding well adapted varieties but to add or substitute a few specific characters such as disease or pest resistance, quality characteristics, herbicide tolerance, lodging or stress resistance. Many of these characters are conditioned by single genes and the job of transferring just a single gene by hybridization, leaving the thousands of other genes unaltered is a very difficult task. This is particularly so when the gene has to be transferred from a widely divergent parent.

The first hope of achieving greater specificity followed the discovery that mutations, heritable genetic changes, could be induced in plants. This led to the hope that mutations could be induced in the specific desired genes, leaving the remainder of the genetic background unaltered. It is certainly true that mutations can be induced in single genes and a large number of useful genotypes and varieties have been produced during the last twenty years. The induction of mutations has been and still is a very useful supplement to plant breeding techniques, but the original hope has not been fulfilled. The induction of mutation remains a random process and as plants have about 50,000 genes, searching for a particular mutation is like searching for the proverbial needle in a haystack. A further difficulty is that after high doses of mutagens, mutations are induced simultaneously at many different loci so the background genotype does not remain unaltered.

CELL CULTURE AND SOMACLONAL VARIATION

The development of techniques for the culture of plant tissue and cells and the regeneration of plants from these cultures has proved a great aid to the selection of improved genotypes where selection can be applied to cells rather than to whole plants.

Initially it was assumed that plants regenerated from tissue culture would be identical with their parent plant and with each other, but this was found not to be so. Research during the 1970s with sugarcane and potatoes revealed considerable variation in a wide range of characters (Heinz, 1973; Shepard et al., 1980). In our own laboratories at Canberra earlier work with sugarcane has been followed by a concentrated effort on wheat and again considerable variation has been discovered.

This variation has been called somaclonal variation (Larkin and Scowcroft, 1981).

Somaclonal variation has now been reported in a wide range of species including many important agricultural species (Scowcroft and Larkin, 1983).

A great variety of characters have been shown to be affected. An extensive evaluation of 65 somaclones of potatoes with acceptable vigour, vine and tuber characters (selected from 1,700 somaclones over two seasons) by Secor and Shepard (1981) showed statistically significant variation in 22 characters. Every somaclone varied from the parent, Russet Burbank, in at least one character and one somaclone differed in 17 characters. Despite this variation the clones resembled the parent variety in general morphology and tuber characters.

In the work from our laboratories with wheat a wide range of characters have also been changed. In addition to head, awn and grain characters and height variation, heritable variation has been shown in maturity time, leaf waxiness and components of the seed proteins (Larkin **et al.**, 1983).

In addition to these morphological and biochemical characters important changes in disease resistance in agricultural species have been reported from various laboratories around the world (See Scowcroft and Larkin, 1983 for review). Enhanced tolerance has been reported to eyespot **(Helminthosporium sacchari)**, Fiji virus, downy mildew **(Sclerospora sacchari)** and smut **(Ustilago scitaminea)** in sugarcane; to early blight **(Alternaria solani)** and late blight **(Phytophthora infestens)** in potato; to southern corn leaf blight **(Dreschslera maydis)** in maize; and to blackleg **(Phoma lingam)** in oilseed rape.

Other potentially valuable traits selected as somaclonal variation and shown to be heritable are tolerance to the herbicide picloram (Chaleff and Parsons, 1978), salt tolerance (Nabors **et al.**, 1980) and amino acid content of seed protein in maize (Hibberd and Green, 1982).

In vegetatively propagated species, such as sugarcane and potatoes the mere presence of stable variation is useful. But in seed propagated species the variability must be heritable to be useful for plant improvement. In the work with wheat somaclones from our laboratory the inheritance and genetic nature of a number of phenotypically distinct somaclonal variants has been followed through a number of sexual generations.

In four of five cases single Mendelian inheritance was demonstrated. In the case of plant height , a character known to be under the control of a number of genes, typical quantitatively inherited selection responses were obtained when selection was applied to selfed second generation progeny (Larkin **et al.**, 1983).

In summary the results from our laboratory and from the work with other species in other laboratories have shown that somaclonal variation

- is heritable
- can be either nuclear or cytoplasmic
- can affect single gene or quantitatively inherited traits
- can be either dominant or recessive
- can sometimes produce homozygous mutants

Thus somaclonal variation is potentially of great value in plant improvement.

However there are still many things we do not know about somaclonal variation.

Is it pre-existing or induced during culture?

Evidence suggests that some is pre-existing, probably more so in cultures taken from terminal tissue, such as leaves, than in those from germ-line tissue, such as immature embryos, but there is extensive evidence that variation is induced during culture (See Larkin and Scowcroft, 1983 for review).

What is the genetic and molecular nature of somaclonal variation?

Both chromosomal and genic changes are known to occur, but the gross chromosomal changes are largely eliminated during sexual reproduction in seed propagated species so they will be of little consequence in plant improvement. But there are some puzzling observations. Dominant changes seem to occur with high frequency which may be a consequence of cell selection systems. Non-segregating homozygous mutants have been reported on a number of occasions from homozygous parents suggesting simultaneous mutation of both loci. Much more research will be necessary before the cause and nature of somaclonal variation will be completely understood.

The apparent high frequency of occurrence?

It is known that genotype and cultural conditions influence the frequency of somaclonal variation. Nevertheless some surprisingly high frequencies have been reported. Evans and Sharp (1983) reported 13 single gene nuclear mutations among 230 regenerated tomato plants (5.7%) and Barbier and Dulieu reported mutation frequencies of 3.5% per locus for two specific loci (a, yg) affecting chlorophyll synthesis in tobacco. Enhanced resistance to disease also occurs at surprisingly high frequency: 15 to 20% for eyespot of sugarcane (Larkin and Scowcroft, 1981); 1% for early blight and 2.5% for late blight of potatoes (Shepard, 1981); and, more than 50% for southern corn blight of maize (Larkin and Scowcroft, 1983).

An even more puzzling feature of the disease resistance studies is the skewed distribution towards increased resistance and the high frequency of resistant variants even in the absence of selection applied to the cell culture (Larkin and Scowcroft, 1983).

GENE TRANSFER

Let me now turn to gene transfer and its role in plant improvement. This really offers the greatest hope of achieving that plant breeder's goal of introducing a single gene, leaving the rest of the genetic background unaltered. But there are still some technical difficulties to be overcome before this goal can be realized.

The opportunities for precise manipulation of the genetic material commenced thirty years ago with the elucidation of the double helical structure of DNA by Watson and Crick. Since then molecular biology has progressed at a fantastic rate so that now we have a great store of information about the nature and function of the genetic material. This includes the determination of the genetic code, which consists of three letter words, coding for individual amino acids, written in a four letter alphabet ATG and C, the nucleotide bases. This message is transcribed from the DNA to the messenger RNA which is in turn translated into proteins.

Probably the most important tools enabling genetic engineering to be performed are the restriction endonucleases. These enzymes recognize specific DNA sequences and cut them in a precise fashion usually leaving 'sticky ends' which permit the 'cutting and stitching' of DNA in a controlled fashion.

Next is the ability to amplify these pieces of DNA. This is done by inserting the pieces of DNA into small circles of DNA called plasmids or into bacterial viruses both of which can multiply in bacteria. Once amplified the piece of plant DNA can then be excised and sequenced.

Recombinant DNA technology or molecular biology was first developed in microorganisms (bacteria and bacterial viruses), then extended to animal systems and now plant molecular biology is fast catching up.

There are some differences between the genes of prokaryotes (the bacteria and viruses) and the eukaryotes (higher plants and animals) but plants and animals seem to have essentially the same organizational features. They each have similar regions which control the start of transcription, the CCAAT and TATAA sequences in front of the coding region. Most plant and animal nuclear genes also have their coding region interrupted by sequences (introns) which are excised during transcription to mRNA.

Now we have the scissors (endonucleases) to cut the genome into small pieces; means of amplifying and sequencing these pieces; and, knowledge that the message from the DNA is transcribed into mRNA. But how do we isolate a particular gene?

Because plants have about 50,000 genes this is not a simple task. It is made even more difficult by the fact that plants contain large amounts of non-coding (repeated sequence) DNA. When the DNA from a plant is cut into 'gene-size' pieces the specific gene has to be searched for among a 'library' of approximately one million pieces.

The method of finding the 'one in a million' is based upon the knowledge that not all genes are equally active all of the time. Some genes are active in response to a specific signal such as a hormone (α–amylase), others are active in specific tissue such as endosperm or cotyledons (seed storage protein), yet others respond to environmental stimulus such as anoxia (Adh).

In our laboratory the Adh gene was isolated by comparing the pools of mRNA from tissue grown in the presence and absence of oxygen (Gerlach **et al.**, 1982; Dennis **et al.**, 1984. Knowing that the Adh gene is induced at low oxygen levels we knew that mRNA would be more abundant in the mRNA pool from the tissue grown at low oxygen concentration. Having obtained the appropriate mRNA, a radioactively labelled DNA copy (cDNA) can be made using the reverse transcriptase enzyme. This cDNA can then be used as a probe to pick the 'one in a million' clones from the genomic library. The mRNA can also be translated in vitro to confirm that the appropriate protein (alcohol dehydrogenase) is produced. In some cases it is now possible to deduce from the amino acid sequence of the product protein the DNA sequence of part of the coding sequence. A labelled polynucleotide can then be synthesised and used as a probe to isolate the gene. If no protein product is known the task is even more difficult but methods are under investigation using mobile genetic elements (jumping genes).

You will be aware that this year's Nobel Prize winner, Dr. Barbara McClintock deduced the existence of such mobile genetic elements in maize by genetic methods long before the era of molecular genetics. Their existence has now been confirmed in microorganisms, **Drosophila** and plants. The system McClintock studied in maize contains two elements Ds and Ac. Ds moves about the genome under the influence of Ac. Ds reveals its position by inactivating the gene where it currently resides.

Thus if we have a probe for a particular gene and know its nucleotide sequence, when that gene is inactivated by the insertion of Ds we can isolate the inactivated gene and deduce the nucleotide sequence of the Ds element. This then opens the possibility of using a Ds probe to locate and isolate another gene which has been inactivated by the insertion of Ds. Because there are many genes in the plant genome the inactivation of any particular gene will be a very rare event and while Ac remains in the system, the Ds-induced mutant will be unstable. However, such Ds-induced mutant can be stabilized by removing Ac.

This system has been used to isolate a Ds element from an Adh mutant that had been inactivated by a Ds insertion. This Ds element is approximately 400 base pairs long and is characterized by 11 b.p. inverted repeats at each end (Peacock **et al.**, 1983).

We can now return to the problem of locating and isolating a gene which does not have a known protein or RNA product, such as a disease resistance gene. If such a gene is inactivated by the insertion of Ds, locating Ds will also locate the gene for resistance. The radioactive Ds probe can be used to locate this region and the DNA sequences adjacent to the Ds region will then be the resistance gene. Research is in progress in our laboratory to use this system to locate and isolate a gene for resistance to rust in maize.

Because mobile elements have the capacity to integrate into the host chromosome they also have the potential for use in gene transfer systems.

For example experiments are also in progress in our laboratory to insert the Adh gene into Ds and to use the Ds element as a vector to achieve transformation in plants by transferring the Adh gene into cells lacking the capacity to produce alcohol dehydrogenase. This has not yet been achieved with plants but similar mobile genetic elements have been used to transform **Drosophila**.

To date the only successful transformation of plants has used a different system, the Ti plasmid of the bacterium **Agrobacterium tumafaciens.**

This plasmid also has the capacity to insert into the host chromosome and in its natural state it introduces oncogenic genes which modify the host plant cells to uncontrolled growth and division and a plant tumour is produced (crown gall). The Ti plasmid has been modified by removing the oncogenic genes and inserting a marker gene into the plasmid (Caplan **et al.**, 1983; Zambryski **et al.**, 1983).

After infection of the plant and insertion of the Ti plasmid into the host chromosome the presence and expression of the marker gene can be detected. Unfortunately this system is restricted to those dicotyledonous host plants, tobacco etc. which can be infected by **Agrobacterium.**

Synthetic vectors are now being constructed and tested which are made up of DNA sequences for replication in bacteria or yeast for amplification; sequences such as the terminal sequences of Ds or the Ti plasmid or recognition sequences from plant nuclear or chloroplast DNA for insertion into the host chromosome; and genes which will respond to selection, such as antibiotic resistance genes.

Attempts have been made to use the DNA plant virus, Cauliflower Mosaic Virus, as a vector for gene transfer but this proved unsuccessful.

Although we have made great progress towards the objective of controlled gene transfer there is still much to be done. In addition to isolating and amplifying the gene and providing it with a mechanism for integration into the host chromosome methods must be devised for getting it into the host cell and protecting it against degradation before integration. Many methods are being tried such as injection, introduction in various forms into plant cell cultures and introduction via germinating pollen. Genes which will respond to selection e.g. antibiotic resistance, are being incorporated and co-transformation methods are being devised and tested.

USEFULNESS OF THESE TECHNIQUES?

These developments are new and exciting but when and how will they prove useful in practical plant improvement?

The pace of development of modern science has accelerated so rapidly over the past decade that it is impossible to predict how quickly new developments will occur. Suffice it to say that ten years ago it took months to determine a short nucleotide sequence of a few base pairs. Now nucleotide sequences of hundreds of base pairs are determined in a few days.

The 'how' is easier to predict. Single gene additions, deletions and alterations will undoubtedly be feasible. Directed mutagenesis, the dream of mutation workers will be achieved by isolating the gene, altering specific base pairs **in vitro** and reinserting the gene. In this way it will be possible to improve the nutritional quality of rice, for example, by increasing the number of lysine residues in the seed storage protein. As we learn more of the gene control systems it will be possible to vary the amount of gene product; to increase the desirable one, to decrease the undesirable ones. The most obvious 'first choices' for effort to be directed will be in the areas of disease and pest resistance, herbicide tolerance, resistance to physiological stress, (heat, cold, salt, drought, etc.) and variation in the amount of biological nitrogen fixation. Exciting as these prospects are, we must not lose sight of the fact that with our current knowledge such manipulations will be confined to single genes. Where selection has to be applied to whole plants, rather than to cells in culture, many of the time consuming and costly constraints which apply to present day plant breeding will still apply. Somaclonal variation is already being extensively tested as an adjunct to existing plant breeding methods. Gene transfer will shortly move from the experimental to the practical phase. Both gene transfer and somaclonal variation will undoubtedly have an impact on plant improvement, but neither will replace conventional plant breeding methods in the immediate future.

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Recent Advances in Biotechnology with Special Reference to Developing Countries

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Biotechnology can be looked upon as the use of cells for making useful products or doing valuable things, with or without genetic engineering being involved. These could be microbial, plant or animal cells. Up to the present time, microbial cells have been by far the most important in this connection but mammalian hybridoma cells, used for the production of monoclonal antibodies, have achieved considerable importance. This paper will briefly refer to the range of products resulting from biotechnology and give a few examples of recent advances that appear likely to influence future progress in this field, especially those which may have a major impact on developing countries.

Table 1 lists the major types of commercially important microbial substances produced by microorganisms. This list excludes the many proteins and polypeptides of non-microbial origin which are beginning to be produced by bacteria and yeasts as a result of recombinant DNA technology. Some of these microbially synthesized compounds are produced in very large quantities. Perlman (1977) cited the annual production of citric acid at over 90,000,000 kg of monosodium glutamate at over 180,000,000 kg, and of the penicillins at over 45,000,000 kg.

Table 1. Microbial Production of Microbial Products

- Organic Acids (about 10)
- Amino Acids (about 20)
- Enzymes (abour 30, excluding lab. usage)
- Antibiotics (over 90)
- Antiparasitic Agents
- Insecticides and Herbicides
- Vitamins and Growth Factors
- Pharmacologically active metabolites
- Solvents and Biofuels
- Single-cell Protein
- Miscellaneous Products

The dramatic effect that the application of both conventional bacterial genetics and gene cloning can have on the yields of some of these products is illustrated by the remarkable yields of alpha amylase now possible with **Bacillus** species. Yoneda (1980) studied a series of mutations each of which increased the yield of alpha amylase by **Bacillus subtilis.** When these mutations were sequentially introduced into a single strain of **B. subtilis** by transformation each added mutation was found to cause a synergistic increase in the amount of alpha amylase produced. Ultimately a 250 fold increase in alpha amylase production was achieved. Another general approach to obtaining increased yields of a bacterial product is to clone the gene encoding that product in an amplifiable plasmid. An amplifiable plasmid is one which exists in multiple copies within a cell and can be induced to form a still larger number of copies by exposure to certain chemicals (such as chloramphenicol) or supraoptimal temperatures. Palva (1982) successfully employed this technique by cloning the structural gene for alpha amylase from **Bacillus amyloliquefaciens** in a plasmid inserted into **B. subtilis.** The amount of α–amylase activity was about 2500–fold higher than that for the wild–type **B. subtilis** Marburg strain and about five-fold higher than the activity produced by the donor industrial–type **B. amyloliquefaciens** strain. The alpha amylase was indistinguishable from that of the donor and virtually all of it was excreted. The increased activity was attributed to a gene dosage effect.

Alpha amylase is one of the enzymes of considerable significance for developing countries because of its ability to convert the starch from tropical root crops, such as cassava, to glucose. It is being increasingly used in combination with the enzyme glucose isomerase to produce sweet "high fructose" syrups. The latter enzyme converts glucose to a mixture of glucose and fructose, which is a much sweeter product than glucose alone. The glucose isomerase is commonly used in the form of intact immobilized cells, with special strains of **Streptomyces, Arthrobacter,** and **Bacillus coagulans** being most common (Johnson, 1979). This is an excellent example of the advantages of using immobilized cells or enzymes for biotechnology processes. This system allows the same enzyme molecules to be used repeatedly as the substrate passes continuously through the reaction column.

Antibiotics

Over 90 antibiotics are produced commercially. Those produced in largest quantity are the penicillins, for therapeutic purposes, but antibiotics for use in animal feeds, to promote growth and feed efficiency, are also produced in huge quantities. A recent advance in this field is the introduction of antibiotics which are for exclusive use in animal feeds and which do not pass into the meat. Many kinds of antibiotics have a dramatic effect in enhancing the growth of livestock and in increasing feed

efficiency. For example, in cattle, the administration of only 70–80 mg of chlortetracycline per day per animal results in an average 6% increase in weight gain and a 4% increase in feed efficiency (Burg, 1982). Since most of the antibiotics which have been used in animal feeds are also used for human therapy, there has been great concern that their use in feeds could promote the build-up of antibiotic-resistant strains. Antibiotic resistance is commonly carried by transmissible plasmids so that antibiotic-resistant enteric bacteria could transmit their resistance properties to pathogenic bacteria. Furthermore, antibiotics such as the tetracyclines are absorbed from the gut and are present in the meat from animals fed on these antibiotics if the proper withdrawal period is not observed prior to slaughter. Antibiotics which are reserved exclusively for use in animal feeds and which are not absorbed from the intestinal tract avoid both of these potential problems. Virginiamycin and bambermycins are two new antibiotics being used exclusively in animal feeds while monensin, also used for animals only, was first used as a coccidiostat in chickens, but was later found to increase the feed efficiency of ruminants by about 10% (Burg, 1982).

Several microorganisms produce products which are active against human and animal parasites. Monensin, referred to above, is produced by a **Streptomyces** species. Secondary metabolites of several **Streptomyces** species have been shown to have antihelminthic activity. An important group of antibiotics called avermectins has such activity and one semi-synthetic derivative, called ivermectin, was reported to be 1,000 times more active than thiobenzote against these parasitic worms (Chabala **et al.**, 1980; Campbell **et al.**, 1983). Searches for anti-parasitic agents have been much less extensive than searches for antimicrobial agents but the former could be very important in developing countries where intestinal parasites tend to be particularly common.

Microbial Insecticides

Microbial insecticides are achieving importance and have special significance for many developing countries. Some 1,500 bacteria, fungi and viruses are potentially useful as insecticidal agents (Miller **et al.**, 1983). Until recently the main interest has been with **Bacillus thuringiensis.** The toxin of most strains of this species is selectively active against insects of the **Lepidoptera** and it is produced commercially. Some strains, however, such as those in the subspecies **Israelensis** and related species like **Bacillus sphaericus,** are active against mosquito larvae. These toxins may prove to be of value in developing countries because of the ban against the use of the most effective chemical insecticides in some countries and the development of insects which are resistant to them, in others. There has been an alarming increase in the incidence of malaria in developing countries in recent years, with about 200 million people afflicted with this disease in a year and about two million deaths per year. Although, as noted later, there is some hope for the development of an effective malaria vaccine, at the present time effective mosquito control remains the best way to control the spread of malaria.

Secondary Metabolites

Many of the secondary metabolities of microorganisms have important pharmacological properties but the search for organisms producing such products has barely begun, largely because there have not been efficient test systems. One such agent which has recently shown a dramatic effect is Cyclosporin A. This compound was originally isolated as an antifungal agent but later, almost accidentally, was found to be an active immunosupressant. It now appears to be the drug of choice for preventing rejection of tissues and organ transplants. Umezawa (1981) identified a large number of microbial products with potential pharmacological activity by looking for their ability to inhibit specific mammalian enzymes. Demain (1983) listed 23 additional enzymes for which specific inhibitors could be useful as drugs.

Biofuels

Biofuels have both present and future potential for developing countries. Microorganisms produce several products which can be used as fuel. These include alcohols, hydrogen and methane. Whereas hydrogen production may someday achieve significance as fuel, biologically produced alcohols and methane are both already contributing significantly to the world's fuel needs.

Ethanol is the alcohol most widely used as a fuel but butanol is also coming under study. By far the largest biotechnology venture is the Brazilian National Alcohol Programme. Brazil is expected to produce about 5×10^9 liters of ethanol this year and the objective is to produce over 10^{10} liters by 1987 (Pimental, 1980, Dixon, 1983). About three quarters of a million cars in Brazil run on 95% ethanol, the rest run on a mixture of absolute ethanol and gasoline. In spite of the large scale and apparent success of the Brazilian alcohol programme. It is clear that an ethanol-based fuel system should not and cannot be adopted by all developing countries. The production of substrate for the ethanol fermentation requires enormous amounts of arable land. Brown (1980) calculated that, depending upon the country, it would require five to ten times as much land to provide ethanol fuel for a car as it takes to produce food for a person. There is a real danger that ethanol fuel programmes could deprive the poor of food while providing motor fuel for the rich. Nevertheless, the development of an industrial society does require motor fuel and in oil–poor countries there could be a place for ethanol as fuel; especially when world petroleum supplies again become scarce.

The traditional procedure for producing ethanol utilizes yeast fermenting sugar. There is a very active interest currently, however, in the use of the bacterium **Zymomonas mobilis,** which is employed in some tropical countries for making various alcoholic beverages, for producing fuel alcohol. **Zymomonas** grows more rapidly than yeast and a given mass of cells produces ethanol more rapidly. It used to be thought that **Z. mobilis** could not produce as high an alcohol concentration as yeast. Some strains, however, tolerate as high an alcohol concentration as most yeasts, with the maximum reported tolerance being 120–130 g alcohol per liter or 15–16% on a volume/volume basis (Rogers **et al.**, 1980). One major advance in **Zymomonas** research is the use of immobilized cells or flocculated cells for continuous fermentation systems. The cells can be immobilized in small beads of calcium alginate (Klein and Kressdorf, 1983) or a flocculating mutant can be used to prevent the loss of cells

with the product (Fein et al., 1983). One problem is that flow rates which give maximum productivity do not yield a very high alcohol concentration. It remains uncertain whether or not the bacterial fermentation system can be developed to be more efficient than yeast for industrial alcohol production.

Cellulose is beginning to be intensively studied as a substrate for fuel alcohol. Several companies are looking at methods for converting wood chips to alcohol (Curtin, 1983) but the technology would be equally applicable to many cellulosic agricultural residues such as sugar cane bagasse. One major recent advance is the use of a steam explosion technique to break up the particles to make the cellulose accessible to hydrolysis by either chemical or enzymatic methods. High cellulose–producing strains of **Trichoderma** have been produced so that one company, Iotech, claims that there is no question that their cellulase process has passed acid hydrolysis in economics. Although no commercial scale cellulose to ethanol process is currently operating, the potential value of such processes is enormous given the large mass of cellulosic waste materials. It is essential to be aware, however, of the potential dangers inherent in the wide-spread use of agricultural residues for such things as ethanol production. It is essential that sufficient organic matter be returned to the soil to maintain soil structure and soil fertility. If fuel alcohol programmes were developed on the basis of removing all agricultural residues from the land, the long-term results could be disastrous.

Butanol is now being looked at in many laboratories as an alternative fuel alcohol because, being a more reduced compound than ethanol, it contains more energy per liter. The major problems here are the intolerance of the producing organisms to high concentrations of butanol, the production of other products, such as acetone and organic acids, along with the butanol, and the strong unpleasant odour of butanol. It seems unlikely that butanol will become a major biofuel in the immediate future.

Single–cell Protein

The use of microbial cells as food or feed continues to be on the borderline of achieving major economic importance. Table 2 lists a few species of microorganisms which may prove to be of significance for single–cell protein production in developing countries. The photosynthetic blue–green algae (or, more correctly, bacterium) **Spirulina** species, have been harvested for centuries as food for people living adjacent to Lake Chad in Africa. These organisms are now grown commercially in Mexico and Israel and have a limited market which includes their sale in speciality health–food stores. Algae or photosynthetic bacteria are particularly favoured by the high intensity and uniformity of sunlight characteristic of most tropical countries.

The Imperial Chemical Industries Process, using the bacterium **Methylophilus methylotrophus** has been operating commercially for several years and was improved about three years ago by genetic engineering (Walgate, 1980). This organism utilizes methanol as a carbon source and normally incorporates ammonia into glutamic acid by glutamate synthase, an energy–requiring process that enables the cells to utilize low concentrations of ammonia.

Table 2. Some Microorganisms of Potential Value as Single Cell Protein in Developing Countries

Blue-green algae/bacteria
Spirulina spp.
Bacteria
Methylophilus methylotrophus (ICI Process)
Yeast
Candida utilis
Candida tropicalis
Filamentous Fungi
Paecilomyces varioti (Pekilo Process)
Fusarium graminearum (Rank/Hovis/MacDougall)
Aspergillus hennebergii (Orstom Process)
Chaetomium cellulolyticum (Waterloo Process)
Cephalosporium eichhorniae (Guelph Process)

During growth in a fermentor, however, relatively high concentrations of ammonia can be used. A gene from **E. coli** which enables the organism to take up ammonia by the glutamate dehydrogenase system, without the use of ATP, was cloned into this SCP strain. This resulted in a significant increase in yield and represents the first practical application of recombinant DNA technology to single–cell protein. ICI has recently designed fermentors which could produce 100,000 tons of SCP per year and the company indicated a particular interest in selling this process for producing Pruteen, as they call their SCP, to Third World countries having a ready source of methane that could be converted to methanol as a substrate for this process (Biotechnology Newswatch, 1983a).

Last year a German company, Hoechst A.G., reported success in extracting potentially harmful materials from **M. methylotrophus** biomass to produce a product, which is principally protein, they named Probion (Biotechnology Newswatch, 1982). Probion is intended for use as a human food and is claimed to be highly nutritious. It is an active emulsifying agent and when mixed with vegetable oil and water produces a margarine–like product in 10 seconds. ICI is now also experimenting with a product for human use, as a milk substitute.

Candida utilis is the commonest food yeast and has often been used in processes utilizing a sugary substrate such as molasses. If starchy substrates are used, however, the starch must first be hydrolyzed to glucose before the yeast can use it. A French company claimed to have a strain of **Candida tropicalis** which hydrolyzes starch (Adour Enterprise, 1981) but few details were given.

Among the filamentous fungi, both the Pekilo process in Finland and the Rank/Hovis/MacDougall process in Great Britain are well developed but the other processes listed in Table 2 may be of more interest for developing countries. The University of Waterloo process uses a wood-rot fungus, **Chaetomium cellulolyticum** to produce SCP from many types of cellulosic materials, including sugar cane bagasse and municipal garbage (Moo–Young et al., 1978). The process requires a minimal pretreatment of

the cellulose and the fungus has a specific growth rate of 0.23 which is unusually high for an organism growing on cellulose. This process has been licensed to several companies for pilot plant production but it is too early to know how successful the process will be in practical use.

The ORSTOM process, developed in France about three years ago, is a solid–state growth system designed to produce protein–rich feed from cassava (Senez et al., 1980). In this process the granulated substrate is sufficiently dry that air can pass between the granules while the fungus invades the granules and produces a product with about 14% protein.

Research at the University of Guelph has concerned a thermophilic fungus, called **Cephalosporium eichhorniae**, grown in aerated liquid cultures with cassava substrate. This process yields a biomass with about 41% crude protein and 31% true protein (Mikami et al., 1982). The advantages of this process are that the organism is amylolytic (so that no prior hydrolysis of the starch is required) and the high growth temperature and low pH make it almost immune from contamination (so that sterilization of the substrate and the maintenance of aseptic conditions are not required). The high growth temperature reduces the cost of cooling the fermentor. All SCP processes utilizing filamentous fungi have the advantage that the biomass can be harvested by a relatively cheap filtration procedure. Extensive feeding trials and sub–chronic toxicity tests with rats indicated that the fungus provided a high quality protein when supplemented with methionine and had no harmful effects in 90–day feeding trials (Alexander et al., 1979; Kuo et al., 1979).

At the present time the relative costs of cassava and protein–rich feeds are not wide enough apart for any SCP process utilizing cassava as a substrate to be economically viable. Roughly speaking, a microorganism uses about one half of its substrate for energy and assimilates the other half into cell material. Assuming that the microbial cells have about the same protein content as soybean meal (which is approximately the case) then it follows that the cost of the substrate (cassava) would have to be less than one half of the cost soybean meal even if a zero–cost conversion process was developed. At present, soybean meal is not twice as expensive as cassava on world markets. If these relative costs change markedly, these microbial processes for producing protein–rich feed from cassava could become important.

Genetic Engineering

The basic technique of gene splicing is simple but the actual procedures are becoming increasingly sophisticated. Basically there must be a vector into which passenger DNA is spliced. This vector is usually a plasmid or a virus. The passenger DNA itself must be isolated, the vector is cut open, the DNA is spliced into the opened vector, and the combination placed in a host cell by transformation. I shall comment briefly upon some of the recent modifications of vectors which permit efficient expression of cloned genes, host–vector systems which permit the product of cloned genes to be secreted directly into the medium, and some recombinant DNA products of particular significance to developing countries.

Expression vectors

A number of vectors have been constructed which allow efficient expression of cloned genes. For example, in **E. coli**, one widely used system employs the control genes for the tryptophan operon. When genes are spliced into the vector immediately following this control region, and placed in a cell which requires tryptophan for growth, then the expression of the cloned genes can be readily controlled. In the normal cellular system, when the cell is in a medium lacking tryptophan the genes coding for the enzymes required for the synthesis of tryptophan are turned on for maximum synthesis of the necessary enzymes. If these genes have been replaced by foreign genes the tryptophan control region now controls the expression of the foreign genes and they are expressed when the cells are placed in a tryptophan–free medium.

Excretion of the Products of Cloned Genes

Until very recently the proteins or polypeptides produced from cloned genes were either retained in the bacterial cell's cytoplasm or, at best, secreted into the periplasmic space. Therefore the desired product was always heavily contaminated with various normal bacterial products. The purification of these products would clearly be greatly facilitated if the desired product were excreted into the culture medium. **Bacillus** species have been considered to be useful hosts for this purpose because they efficiently excrete a large number of proteins.

The recent cloning of alpha–amylase genes in **Bacillus subtilis** by Palva (1982), referred to earlier, enabled the control region for this gene to be used in vectors for gene cloning in this organism. This control region includes not only an efficient promoter for expression of the cloned genes but also a sequence which codes for a sequence of amino acids at the amino terminus of the enzyme and permits it to be secreted from the cell. This sequence is termed a secretory signal sequence. Palva et al. (1983) placed the gene for human alpha–2 interferon following the promoter and signal sequence for the alpha–amylase gene in **B. subtilis** and found that interferon was, in fact, excreted from the cell.

E. coli, the most common host species for gene cloning, was considered to be unable to excrete proteins into the medium although it secreted some proteins into the periplasmic space. Recently, however, there have been results indicating that **E. coli** can excrete some proteins. Hemolytic strains of **E. coli** produce a hemolysin which may be excreted into the medium (Biotechnology Newswatch, 1983b) so it is possible that cloned genes could be spliced to whatever control system permits this excretion. When the gene for a cellulase from a thermophilic bacterium, **Thermomonospora YX**, was cloned in **E. coli**, about 30% of the endoglucanase activity appeared in the medium, about 30% in the periplasmic space, and the remainder was in the cytoplasm (Collmer and Wilson, 1983). In another laboratory, a so–called "secretion gene" was cloned in **E. coli** which caused an extensive secretion of several proteins into the medium (Biotechnology Newswatch, 1983c).

Yeasts are rapidly gaining favour as cloning hosts because they have the ability to excise the introns, or

non–coding sequences which interrupt the coding sequence in many eukaryotic genes. They appeared, until recently, however, to share with **E. coli** the apparent problem of not being able to secrete the products of cloned genes into the medium. Hitzeman **et al.** (1983) found that human interferon genes containing secretory signal sequences, when cloned in **Saccharomyces cerevisiae**, resulted in the secretion of up to 21% of the interferon activity into the medium. Merryweather **et al.** (1983) also succeeded in having yeast cells export foreign cloned polypeptides. Their success was based on the fact that haploid yeast cells of the α–mating type secrete a peptide mating pheromone called the α–factor. Cloned genes were placed on a plasmid immediately following the region coding for the α–factor leader region plus a short spacer region. Large quantities of insulin and other gene products were secreted in this way – reportedly in gram quantities.

Vaccine Production via Recombinant DNA

Several viral antigens and several antigens of malarial parasites have been cloned. Vaccines for Foot–and–Mouth Disease virus (Kleid **et al.**, 1981) and Hepatitis B (Biotechnology Newswatch, 1983d) produced by **E. coli** from recombinant DNA are now undergoing field trials. The cloning of antigens from these two viruses both present novel features. The Foot–and–Mouth Disease virus has an RNA genome but since all cloning systems require DNA it was necessary to produce a DNA copy of the viral genome prior to cloning it. The Hepatitis B virus story is particularly dramatic because this virus cannot be cultivated in cell culture in the laboratory. Nevertheless, enough DNA was obtained, from virus particles in the blood of infected patients, to be cloned, and now large amounts of Hepatitis B coat protein can be produced in bacteria. Both of these vaccines, if successful in producing high levels of immunity, should be of particular value in those developing countries in which these diseases are endemic.

Effective vaccines for malaria are probably still someway down the road but some remarkable strides are being made. The problems are formidable because the four major stages of the parasite all have different antigens. Furthermore, the antigens encountered are known to vary in different strains of the same species (Cox, 1983). Researchers at the New York University Medical Center and the National Institutes of Health in the USA reported cloning the one major antigen of the sporozoite form of **Plasmodium knowlesi** from monkeys (Ellis, 1983). Kemp and his colleagues in Australia have cloned several antigens from the blood stages of **Plasmodium falciparum** one of the four malaria parasites affecting human beings (Kemp, 1983). The techniques were essentially similar. Messenger RNA was isolated from the desired stage of the parasite, a DNA copy of the RNA was made and this mixture of DNA molecules was cloned in **E. coli**. In one case monoclonal antibodies were used to detect clones carrying the gene for the desired antigen. It remains uncertain how effective malaria antigens synthesized by bacteria may be in conferring immunity to malaria.

One of the most exciting prospects for the production of vaccines is the cloning of antigens from various infective agents in the vaccinia virus. The vaccinia virus has been used for 187 years, since Edward Jenner first developed the use of cowpox virus for immunizing against small pox. It has been so effective as a vaccine against small pox that small pox has been totally eliminated from the world. Now it seems it is possible to attach the gene for virtually any antigen to a gene for a vaccinia virus surface antigen and produce a form of vaccinia virus which could induce immunity to the cloned antigen. Ultimately, modified vaccinia virus carrying antigens for several different pathogens, including the hepatitis B virus and the malaria parasites, will probably be available. For those vaccines which prove to be effective, this method of delivery will be very cheap. Certainly effective vaccines for hepatitis B and/or malaria would have profoundly beneficial effects in developing countries.

Genetic Engineering of Plants

The prospects of successfully using recombinant DNA in higher plants are rapidly improving. The three major cloning vectors involved are the cauliflower mosaic virus, which is unusual among plant viruses since it is a DNA–containing virus; shuttle vectors in which chloroplast DNA is combined with an **E. coli** vector; and the Ti plasmid of **Agrobacterium tumefaciens**. The Ti or tumour–inducing plasmid seems to be the most promising (Schell and Van Montagu, 1983). **Agrobacterium tumefaciens**, the cause of crown gall disease, has developed its own procedure for genetically engineering its host plant. It carries a plasmid which is transferred to plant cells and a portion of this plasmid, called the T DNA, becomes inserted in the plant genome. One of the genes in the T DNA causes the plant cell to produce an unusual amino acid, either nopaline or octopine. The same plasmid codes for the information which allows the crown gall bacteria to utilize the unusual amino acid synthesized. Thereby the bacterium has evolved a unique ecological niche for itself. The control sequences for the synthesis of these unusual amino acids can be used to promote expression of the cloned genes placed immediately after them.

J.D. Kemp and T.C. Hall (see Netzer, 1983) recently reported the expression in tobacco and sunflower plantlets of the gene for phaseolin, the major storage protein in beans. This is the first reported success in obtaining foreign gene expression in whole plants. The pathway to applying recombinant DNA techniques to the improvement of agricultural plants appears to be open.

Genetic Engineering of Animals

Some progress has recently been made in the genetic engineering of animals. The most dramatic was the creation of giant mice by injecting cloned rat growth hormone genes into newly fertilized mouse eggs (Palmiter **et al.**, 1982). The grown hormone gene had been placed next to the promoter region for mouse metallothionein. Similar results were obtained by placing the gene for human growth hormone in mice (Palmiter **et al.**, 1983). It is by no means clear whether more rapid growth of farm livestock can be achieved by similar experiments or whether this will be desirable. Nevertheless, the route to applying genetic engineering to domestic animals for the benefit of mankind is also opening up.

CONCLUSION

Biotechnology is rapidly advancing in many directions.

Although many of the more dramatic advances have been in the development of pharmaceutical products, via recombinant DNA technology, which do not have special relevance for developing countries, there are several recent discoveries which are of major significance for such countries. To a large extent the advances possible in this field are limited by our own imaginations. In order for biotechnology to achieve its full potential in developing countries it is essential that research in biotechnology be strongly supported in these countries. The needs of these countries and the ways in which biotechnology can help meet these needs can best be perceived by trained scientists working in these countries.

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Dr Chua Sian Eng, President, Singapore Institute of Biology, addressing the gathering.

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From left to right – Dr Chua Sian Eng, Dr Tay Eng Soon, Prof S Radhakrishna, Dr Khoo Hong Woo.

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The Application of Recombinant DNA Techniques for the Study of Human Genetic Diseases

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ABSTRACT

The technique of restriction gene mapping has been developed locally for the detection of carriers and at risk unborn foetuses for α – thalassaemia. However, for most of the β – thalassaemias where gene deletion is not involved, this approach is not applicable. The diversity of the molecular defects for β – thalassaemia preclude the use of any simple method for detection. On the other hand, the use of DNA polymorphism coupled with linkage analyses within the β – globin gene cluster may provide an alternative. In this study, we analysed the restriction enzyme pattern at seven polymorphic sites (i.e. haplotypes pattern) among Chinese thalassaemics in Hong Kong where the disorder is prevalent. Distribution of the haplotypes is non–random. The frequency of the haplotypes is different from that of the Mediterranean population. Furthermore some new haplotypes have definitely been identified among the Chinese. Cloning and DNA sequencing of some of the thalassaemic alleles associated with these haplotypes are in progress.

INTRODUCTION

The use of restriction endonucleases for molecular gene mapping coupled with gene cloning, DNA sequencing and assays of gene expression in heterologous systems have launched the study of many human diseases to the molecular level. The haemoglobinopathies including the thalassaemias are among the first few human inherited disorders to have been studied to such details. In the first part we report that the relevant techniques have been established locally and can now be applied for α and some of the β–thalassaemias, both of which are prevalent among the Southern Chinese. For the majority of α–thalassaemias where gene deletion is involved, simple restriction mapping, which depends on the type and size of the DNA fragments produced, can be used to differentiate the various genotypes.

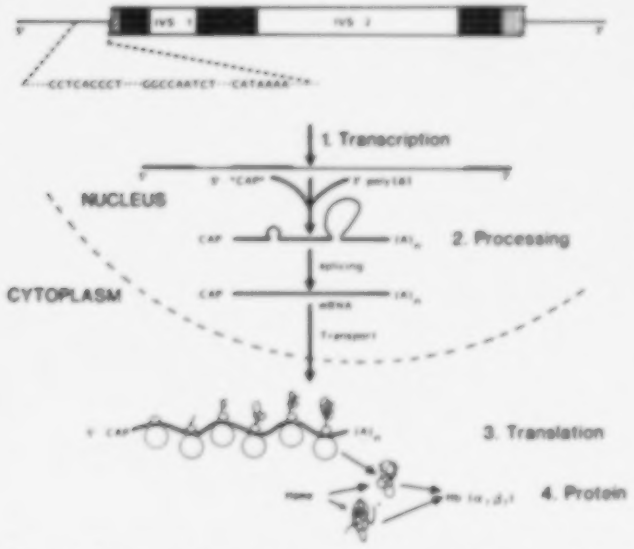

Figure I: Diagram illustrating the various stages from a gene (specific DNA sequence) to the gene product (protein). Mutation, in the DNA, which affect any one of the stages could result in a decreased amount of β–globin polypeptide.

However the situation with β–thalassaemia is more complicated. Often the phenotypes are grouped as β^+ or β^0 on the basis of reduced or total absence of β–globin production. During the past few years, Trecartin et al (1981), Treisman et al (1982), Poncz et al (1982) and Orkins (1982 a & b) have identified several types of β–globin gene defects. These include mutations which affect anyone of the following processes: transcription from DNA to mRNA; mRNA processing; translation from the mRNA to protein and assembly, transport or further modification of the protein molecule (Fig. 1). Thus the diversity of the molecular defects of β–thalassaemia preclude the use of simple restriction mapping for the detection of genotypes. The exceptions being the work of Kan et al (1978) and (1979) for the detection of sickle cell anaemia and β–thalassaemia respectively; and that of Kazazian et al (1979) for δ–thalassaemia.

Although linkage of certain polymorphic sites around the β–globin gene have been identified to be associated with specific defects among some ethnic groups, this approach may not be applied universally. Thus the identification of specific novel mutations would still have to rely on molecular cloning and DNA sequencing of the alleles. It is non–practical to perform such tasks indiscriminately. Thus we attempt to apply the use of polymorphic restriction sites within the β–globin gene cluster (Fig. II) in order to provide a more systematic and realistic means of delineating various β gene mutations.

In addition to the Bam HI site, 3′ to the β–globin gene and the two Hind III sites in the γ^G and γ^A genes, three Hinc II sites and an Ava II site have been looked at. Each particular combined cleavage pattern of the seven restriction sites is defined as a haplotype. Particular thalassaemic allele may be associated with specific haplotypes or otherwise. Thus it is necessary to identify the common haplotypes and their frequencies within an ethnic population. The basis of this study is aimed at these ends among the Chinese in Hong Kong.

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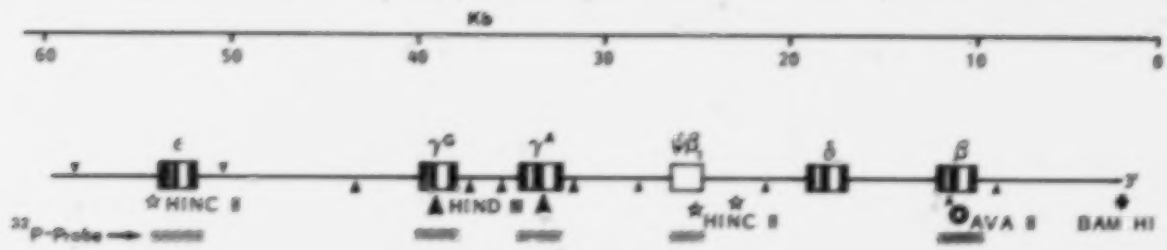

Figure II: Linkage map of the β–globin gene cluster indicating the seven polymorphic restriction sites. Non–polymorphic sites (▲); Polymorphic sites – Hinc II (▽); Hind III (▲); Ava II (○) and Bam HI (◆).

MATERIALS AND METHODS

Genomic DNA was isolated from the leukocytes of 10 ml of EDTA-treated peripheral blood or from cultured amniotic fluid cells as described by Kunkel et al (1977). The samples were obtained from unrelated individuals, of Chinese origin, affected with either α or β–thalassaemia. Each DNA sample (10–15 μg) was digested with various restriction enzymes, using conditions recommended by the commercial suppliers. Digestion was assessed by running small aliquots on the minigel. Generally the digestions were carried out overnight. When digestion was completed, the DNA fragments were separated by electrophoresis in 0.8% agarose gel, transferred to nitrocellulose membranes by the method of Southern (1975) fixed and hybridized with the relevant [^{32}p] – labelled probes as described by Scotts (1979) and Jeffreys (1977). Filters were washed and autoradiographed as described by Scotts (1979), using Kodak X–Omat AR films with intensifers. After treatment with Denhardt solution at 70°C for 2 hrs to remove bound [^{32}p] – labelled probes, the filters could be reused and hybridized with a new probe. This was the case for DNA samples digested with Hinc II. The filters were hybridized with labelled ξ–globin genomic sequences; after extensive washing, were then hybridized with $\psi\beta_1$ genomic probe.

Preparation of radioactive probes

Five probes were used: (i) a 1.8 kb Mbo II DNA fragment derived from the recombinant plasmid JW 101 containing human α cDNA sequencies. (ii) a 1.65 kb Hha I DNA fragment derived from the recombinant plasmid JW 102 containing human B cDNA sequences (iii) a 1.60 kb Hha I DNA fragment derived from the recombinant plasmid JW 151 containing human γ cDNA sequences (iv) a 1.8 kb Bam HI/EcoRI DNA fragment derived from the recombinant plasmid TεG 51, containing human ξ–globin genomic sequences (v) a 1.7 kb Bg1 II/Xba I DNA fragment derived from the recombinant plasmid p3E7.2 containing human $\psi\beta_1$ genomic sequences. Nick translation was carried out as described by Wilson et al (1978) using [^{32}p] dCTP & dTTP as substrates and E. coli DNA polymerase I.

All experiments involving recombinant DNA were performed in accordance with the National Institute of Health guidelines.

RESULTS AND OBSERVATIONS

Detection of α–thalassaemics genotypes

When genomic DNA from α–thalassaemics or normal individuals was digested with the enzyme EcoRI (which recognises the sequence G$^{\downarrow}$AATTC) and hybridized to the α DNA probe, different size fragments could be identified corresponding to the number of α–globin genes on each of the two chromosomes present. The type and size of the DNA fragments produced by each of the genotypes are shown in Fig. III. The techniques for this approach has been successfully developed locally and can now be used for the detection of carriers particularly genotypes 4 & 6. Though the band pattern produced were identical for genotypes 1 & 4, they could be differentiated from the intrinsic intensity of the signal corresponding to the number of α–globin genes present in the entire genome.

Similarly when DNA was isolated from cultured amniotic fibroblasts, no DNA fragment hybridized with the α–globin probe with the genotype 7 which was responsible for hydrops foetalis (Figs. III & IV). The absence of any band (Fig. IV) could thus be used for the positive prenatal diagnosis of such inherited defect.

A similar pattern of DNA fragments, though the actual size would differ, would be observed when the enzyme Bam HI was used instead.

β– thalassaemias

When DNA from a number of β–thalassaemia patients was digested with EcoRI and hybridized to the appropriate β–globin probe, three or four fragments were identified (Fig. IV). These fragments were produced as a result of the EcoRI site within the β–globin gene and that within the δ–gene. However, the digestion patterns were no different from that obtained from a normal individual (Fig. IV). The exception was the pattern obtained from a patient with hereditary persistence of foetal haemoglobin (HPFH). No DNA fragment corresponding to the β–globin gene was observed.

Haplotype identification

Hinc II polymorphism

When the geomic DNA from various β–thalassaemics was digested with the endonuclease Hinc II and hybridized with [^{32}p] ξ–globin gene probe, we observed either a nucleotide sequence of 3.7 kb and/or another fragment of 8.0 kb. Presence of the Hinc II polymorphic site at 0.5 kb 5′ from the ξ–globin gene gave rise to a 3.7 kb

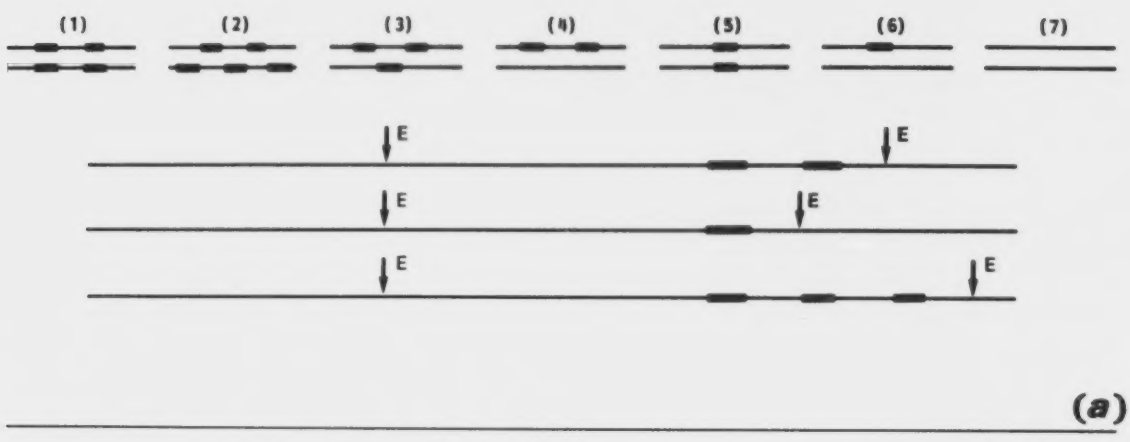

Figure III (a): EcoRI restriction sites around the human α–globin genes. (b) Restriction fragments identified with an α cDNA probe. The pattern corresponds to each of the genotypes shown in (a).

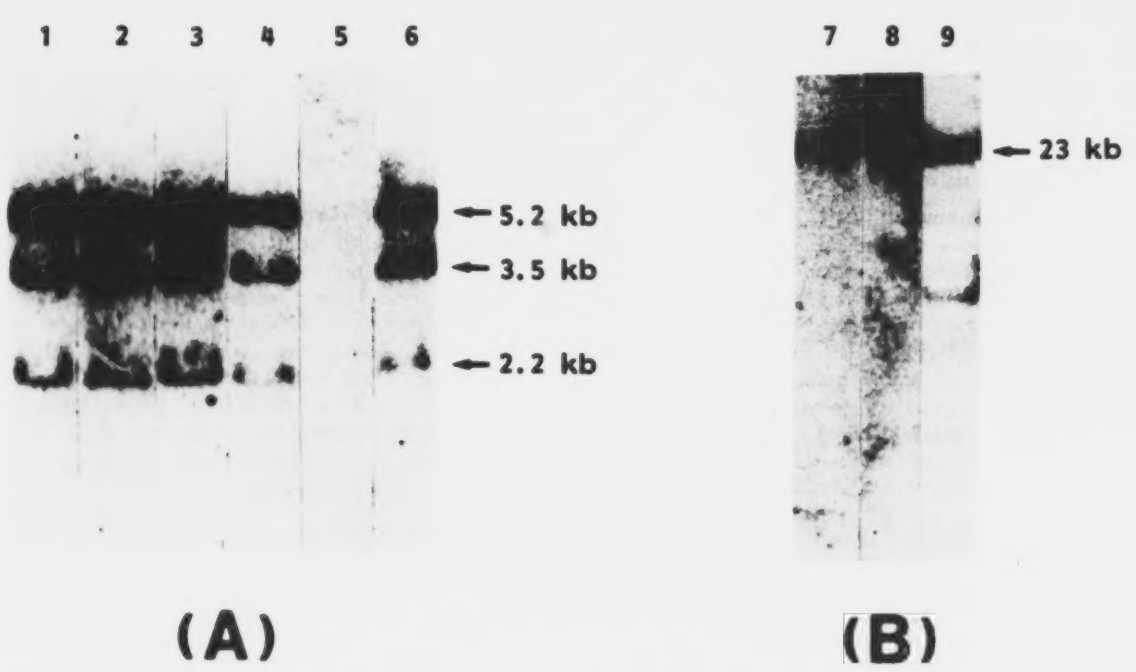

Figure IV: Restriction endonuclease (EcoRI) analysis of DNA isolated from thalassaemic and normal individuals. The DNA was digested, blot transferred and hybridised as described in text.

(A) β–[^{32}P]cDNA probe (B) α–[^{32}P]cDNA probe;

Lanes 1 and 9 – normal individuals, lanes 2 and 4 – β^+ thalassaemia homozygotes; lanes 5 and 7 – homozygous HPFH; lane 6 – β° thalassaemia heterozygote and lane 8 – homozygous α thalassaemia (hydrops foetalis).

1600 – 1615 Effects of mining discharge to phytoplankton
– Saraya, A., Brackishwater Fisheries Division, Department of Fisheries, Bangkok, Thailand

1615 – 1630 Dissolved organic matter in the Veller estuary
– Balasubramaniam, T., India

1630 – 1645 Taxonomy and seasonal fluctuations of Cladocera (Crustacea: Branchiopoda) of River Krishna near Vijayawada, South India
– Prasad, M.K.D. & Y. Radhakrishna, Nagarjuna University, India

1645 – 1700 Preliminary studies on the isolation and culture of marine yeasts
– Tech, E. et. al., SEAFDEC Aquaculture Department, Philippines

AQUACULTURE

Friday, 4 November 1983

AQUACULTURE I
Chairman: Dr L.M. Chou
Conference Room 1

1330 – 1345 Effect of stocking density and the growth and survival of **Trionyx sinensis** hatchlings
– Choo, B.L. & L.M. Chou, National University of Singapore, Singapore

1345 – 1400 Live animal food for rearing carp fingerlings in aquaculture
– Murugan, N., Madura College, India

1400 – 1415 Polyculture of Indian prawn **Penaeus indicus** (H. Milne Edwards) and milk fish **Chanos chanos** (Forskal) in ponds at Pitipana, Sri Lanka
– Jayasinghe, J.M.P.K., National Aquatic Resources Agency, Sri Lanka

1415 – 1430 Optimum conditions for mass culture of **Rana tigrina** tadpole
– Pandian T.J. & M.P. Marian, Madurai Kamaraj University, India

1500 – 1530 Tea/Coffee Break

AQUACULTURE II
Chairman: Mr G. Tay
Conference Room 1

1530 – 1545 Contributions to the culture methods of **Tubifex tubifex**
– Marian, M.P. & T.J. Pandian, Madurai Kamaraj University, India

1545 – 1600 Mixed culture of milkfish and prawns – a more profitable venture
– Nandakumar, R. & V.K. Venugopalan, Annamalai University, India

BIOCHEMISTRY, PHYSIOLOGY & GENETICS

Thursday, 3 November 1983

BIOCHEMISTRY, PHYSIOLOGY & GENETICS I
Chairman: Dr C.L. Poh
Conference Room 4

1045 – 1100 Laboratory studies of Anabaena **flos-aquae** (Lynab.) Bred., a bloom-forming algae isolated from an eutrophic lake
– Chiam-Tai, Y.E., National University of Singapore, Singapore

1100 – 1115 Alcohol dehydrogenase polypeptides (ADH) and waterlogging tolerance in maize
– Chow, K.H., National University of Singapore, Singapore

1115 – 1130 Control of ethylene production by auxin transport inhibitors
– Kang, G.B., Yonsei University, Korea

1130 – 1145 Effects of plant growth substances in callus and organ formation in potato tissue culture
– Chadha, Y.V., Ewing Christian College, India

1145 – 1200 Small angle scattering studies on chondroitin sulphate
– Seshasayee, M., IIT, Madras, India

1200 – 1215 Phenophase calenda, breeding system and reproductive effort of **Striga augustifolia** (don) saldanha
– Rao, P.N. & B.V.N. Reddy, Nagarjuna University, India

1215 – 1330 Lunch

fragment while its absence would give an 8.0 kb fragment (Fig. Va). Both types of fragments could be detected in a heterozygous individual.

When the nitrocellulose paper, was washed and rehybridized with a $\psi\beta_1$-globin gene probe, different fragments of the following sizes: 3.0; 4.6; 6.0 and 7.6 kb could be observed. As there are two polymorphic Hinc II sites, one within the $\psi\beta_1$ globin gene and one 3' to the $\psi\beta_1$ globin gene (Fig. II), there can be a large number of genotypes. The absence of both polymorphic sites (––) haplotype produced the 7.6 kb fragment; presence of only the 3' site (– +) gave a 6.0 kb fragment; presence of only the 5' site (+ –) yielded both 3.0 kb and 4.6 kb fragments while the presence of both sites (++) yielded two fragments of the same size 3.0 kb.

Hind III sites

There are two Hind III sites, one in each of the IVS–II of each of the γ genes. When human genomic DNA from

Table 1

5' ----------DNA----------3'

Globin genes	ϵ	γ^G	γ^A	$\psi\beta_1$		β	
Polymorphic sites							
DNA fragments	Hinc II (+) 3.7 kb	Hind III (+) 7.2	Hind III (+) 2.7	Hinc II (+) 6.0	Hinc II (+) 2.0	Ava II (+) 2.0	Bam HI (+) 9.3
Frequency							
Mediterranean	46	36	23	13	29	87	80
Hong Kong Chinese	75	10	15	16	16	27	75

Frequencies of the polymorphic restriction sites among the Hong Kong Chinese β–thalassaemic patients. Data was obtained from analysis of 40 human chromosomes.

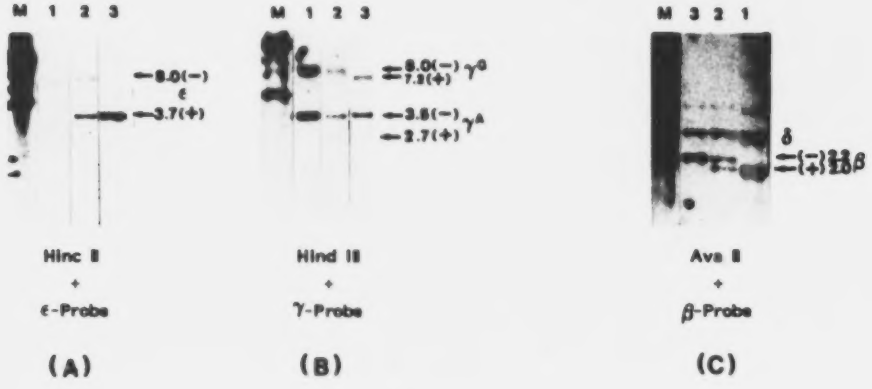

Figure V: Autoradiograms of restriction endonuclease patterns produced by Hinc II, Hind III and Ava II polymorphisms.
(a) Genomic DNA was digested with Hinc II and hybridized with the ϵ–globin gene probe. Lane M – size marker; Lane 1 and 3 – are heterozygotes for the restriction site; lane 2 – homozygote.
(b) Genomic DNA was digested with Hind III and hybridized with γ–probe
(c) Genomic DNA was digested with Ava II and hybridized with β probe.

various β–thalassaemics was digested with the enzyme Hind III (which recognizes A↓AGCTT) and hybridized to the γ cDNA sequences, presence of the Hind III site in γ^G gave a 7.2 kb fragment while its absence gave a 8.0 kb fragment instead. At the same time, this probe hybridized to γ^A gene. A 2.7 kb fragment was detected instead of a 3.5 kb fragment when there was a polymorphic restriction site in the γ^A DNA (Fig. Vb).

Ava II site

In the same manner, when genomic DNA was digested with Ava II (which recognizes the sequence 5'GG ($\frac{A}{T}$) CC3') and hybridized with a β–globin cDNA probe, a DNA fragment of either 2.0 kb or 2.2 kb was observed depending on whether the polymorphic Ava II site, within the IVS–II of the β–globin gene, is present (Fig. Vc).

Bam HI site

Digestion with Bam HI and hybridization with a β–globin gene probe would identify a fragment of 9.3 kb or 22.0 kb depending on the presence or absence of the site, 3' to the β–globin gene.

Each combined pattern of polymorphic restriction sites is defined as a haplotype. Theoretically with seven polymorphic sites, there should be 128 possible combinations. In our preliminary analysis with forty chromosomes, from Chinese β–thalassaemia patients, the haplotypes identified fell far short of the number expected. Furthermore, some haplotypes are found more frequently than others, with haplotype (+------+) being the most frequent. This suggests that distribution of the haplotypes is non–random.

In the 40 chromosomes analysed, the frequency of each of the seven polymorphic restriction sites is shown in Table I. The frequency found at some site i.e. Hind III at the γ^G gene and Ava II at the β–gene, among the Chinese was significantly different from those reported by Orkin et al (1982a) for the Greek and the Italian. The result suggests that the frequency of the haplotypes found in the Chinese would be expected to be different from the frequency found in the Mediterranean population. This was indeed reinforced by the approximate frequency of the haplotypes found in the Chinese β–thalassaemia patients. The haplotype (+------+) being the most common with an approximate frequency of 40% but was reported by Orkin (1982a) to be present in only 6% among the Mediterranean population. On the other hand, haplotype (+----++) was found in only 10% of the Chinese studied while it was the most commonly occurring among the Greek and the Italians.

In the absence of (unavailable) family data, whereby in many instances, the haplotype could not be definitely ascertained. However in other cases e.g. an individual typed as (±-----+), the haplotypes could only be (+-----+) and (------+). From this sort of analysis, we have identified the new haplotype (------+). In the same way, we have so far identified a few more new haplotypes e.g. (+------) and (-----+–) which have not yet been reported for other ethnic groups.

DISCUSSION

The successful development of the restriction mapping technique locally means that we can now employ this for identification of the genotypes for genetic counselling of α–thalassaemia. At the same time, it can be adopted for the pre–natal diagnosis of at risk pregnancies for homozygous α–thalassaemia. The latter is not an uncommon condition in this part of the world, as the frequency of carrier has been estimated to be 3–5% among the Southern Chinese.

This same method by Tam et al (1980) was also used to delineate the mechanisms involved and showed that gene deletion was responsible for many of the hereditary persistence of foetal haemoglobin. However, because in most of the β–thalassaemias gene deletion is not involved, and that the molecular mechanisms seem to be diverse as reported by Trecartin et al (1981), Treisman et al (1982), Poncz et al (1982) and Orkins (1982 a & b), simple restriction gene mapping cannot be used for this group of disorders as shown by Tam (1980). Pirastu et al (1983) used short synthetic oligonuclectide sequence (spaning the region around the point mutation at codon 39) homologous to the β–thalassaemia gene, common among the Sardinians as the probe to circumvent this difficulty.

Linkage to polymorphic restriction sites within the β–globin gene cluster reported by Orkin (1982a) and Antonarakis (1982) seems to provide an alternative approach. The results from this study suggest that distribution of the patterns of restriction site polymorphism i.e. haplotypes is non–random and this is in accordance with the findings of Antonarakis (1982) for other ethnic groups. Use of this special feature can be envisaged. Linkage of each of the common haplotypes to a particular β–thalassaemic allele or the β^A allele would be the most ideal situation. This would mean the use of haplotypes, as in conventional linkage of a marker gene to a gene responsible for a diseased condition, in the pre–natal detection of the severe condition. Though the β–thalassaemic allele with an amber mutation in codon 39 (common Mediterranean allele) was shown by Orkin (1982a) to be coupled to the same haplotype in three separate families, this strict adherence might not be always observed. Thus it is necessary to identify the common haplotypes and their linkage to specific β–globin gene mutations for any particular ethnic group. Restriction site polymorphism and haplotype identification are comparatively easier than cloning and sequencing every possible β–thalassaemic allele at random.

The findings of new haplotypes among the Chinese patients together with the significantly different frequency of some of the haplotypes, from that reported by Orkin (1982a) for the Mediterranean population suggest that with this systematic approach, we may be able to identify novel mutations in the β–globin gene among the Chinese who are phenotypically grouped as β^0 or β^+ thalassaemic. In fact, Orkin et al (1983) used this strategy, cloned and identified a mutation in the "ATA" box, in a Chinese β–thalassaemic patient who was homozygous at the polymorphic restriction sites examined.

Some of the β–thalassaemia alleles associated with the haplotypes identified, are being cloned and sequenced. We hope the findings would be forthcoming. These would lead to more detail molecular description of some of

these mutations. Should some of the β mutations be tightly linked to particular haplotypes among the Chinese, together with family data, information on haplotypes may be used to predict the presence or absence of β–thalassaemic alleles.

This approach of haplotypes being coupled to specific mutation of a single gene locus has been adopted for the characterisation of other inherited diseases e.g. insulin (Rotwein 1983); analbuminemia (Murray 1983), and could be extended to others.

Note added in Proof

This paper was presented and submitted for publication in November 1983. Since then we have analyzed over 150 chromosomes from thalassaemic and normal individuals. In addition to the new haplotypes reported in this paper we have identified some more, including those recently reported by Cheng, T.C. et al. Proc. Natl. Acad. Sci. (USA) 81, pp 2821–2825, May, 1984. The types and frequencies of the haplotypes identified in the Chinese (detail data will be reported elsewhere) are distinctly different from those of other ethnic groups as reported by Beldjord, C. et al; Human Genetics 65:204–206 (1983); Kazazian, H.H., The EMBO Journal 3: 593–596 (1984) and Antonarakis, S.E. et al.; Proc. Natl. Acad. Sci. (USA) 8:1154–1158 (1984).

ACKNOWLEDGEMENTS

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Brem Bali Fermentation*

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ABSTRACT

Brem Bali is a traditional alcoholic drink from the island of Bali. The substrate used is glutinous rice of either white or black variety. A study was conducted on the fermentation process using a commercial inoculum which contained the mould **Mucor indicus** and the yeasts **Candida parapsilosis**. From the third day the "tape" juice could be collected. The highest amount of juice obtained was after 5 days of incubation at ambient temperature. The concentration of the inoculum was 0.375% (w/w). The changes occurring in the substrate during the fermentation process were studied. The residue which was left after separation of the juice from the substrate still contained some nutrients, the composition being moisture 58.6%, protein 4.3%, fat 0.9%, carbohydrate 37.7%, ash 0.25% and vitamin B_1 0.004 mg/100 grams of sample. The residue is now utilized as a snack.

INTRODUCTION

Brem Bali is a traditional alcoholic drink from the island of Bali. The Balinese prepare it for their holy ceremonies or for traditional medicine and at present it is served also as a welcome drink for tourists visiting the island of Bali. The taste is light sweet–alcoholic. The colour depends on the glutinous rice used as substrate. It is light yellow if the white variety is used and dark liver–red if the substrate is of the black variety.

The inoculum used is a commercial "ragi tape" made from rice flour and it is imported from Central Java. Until today various qualities of home made Brem Bali can be found at the markets.

The instability of the quality of Brem Bali was one of the reasons for this study.

MATERIALS AND METHODS

Substrate

Glutinous rice of the white and black variety of good quality were bought from a market in Jakarta. The rice was washed and cooked with water, the ratio of rice: water being 1:1 (w/v) for the white variety and for the black variety, 1:1.5 (w/v). They were steamed for 20 minutes, then spread on a sterile stainless steel tray for cooling.

Inoculum

Two kinds of commercial "ragi tape" used in this study were Roda Mas (code R) from Central Java and Semar (code S) from Western Java.

Inoculation

To examine the production of juice, the cooled steamed rice was inoculated with both "ragi tape" at a concentration of 0.25%, 0.375% and 0.50% (w/w). The inoculated substrate was put in sterile wide mouth Erlenmeyer flasks and closed tightly with aluminium foil.

To study the changes accuming in the subtrate during the fermentation process of 6 days, only "ragi tape" R at a concentration of 0.375% (w/w) was used.

Examinations were carried on the pH, aroma, consistency of the substrate, the amount of juice produced, and consistency of the residue after separating of the juice from the substrate.

Analysis

Changes of the total solids, total acids and amino nitrogen occurring in the substrate were examined using the method of Sorensen (AOAC, 1975). The soluble carbohydrate content was determined using the anthron method, while the alcohol content in the juice was measured following Pearson (1941).

RESULTS AND DISCUSSIONS

Ragi tape R contained (22–29) x 10^6 microorganisms per gram. The mould was identified as **Mucor indicus** and the yeast as **Candida parapsilosis** (Ashford) Langeron and Talice. Ragi Tape S contained (55–72) x 10^6 microoganisms per gram. The mould isolated from this ragi was **Mucor indicus**, but the yeast was identified as **Saccharomycopsis fibuligera** (Lindner) Klockner (Lodder, 1971).

Both ragi tape R and S yielded the specific tape taste after 3 days of incubation for the white glutinous rice variety inoculated with 0.25% (w/w) inoculum, and after 2 days for the black variety of glutinous rice. For both kinds of glutinous rice 0.375% (w/v) inoculum produced the specific tape taste after 2 days of incubation. With 0.50% (w/w) inoculum a dry less juicy tape with an unpleasant aroma and taste for both substrate was produced.

After certain incubation time (days) the tape was

Table 1. Juice production (ml) from 50 grams of fermented glutinous rice with various concentrations of ragi tape R and S.

Ragi tape (%)	Fermentation time (days)	Glutinous rice			
		White variety		Black variety	
		R	S	R	S
0.25	3	34.6	16.0	48.0	44.6
	4	53.0	34.3	53.0	49.0
	5	57.3	37.3	53.3	51.3
	6	59.0	51.0	55.0	52.0
0.375	3	55.3	35.6	39.3	40.3
	4	60.6	45.0	40.0	41.6
	5	67.0	57.3	44.0	50.3
	6	63.3	58.6	46.6	47.0
0.50	3	25.6	33.1	13.0	–
	4	35.0	52.0	17.0	13.3
	5	37.0	52.3	30.3	29.0
	6	35.3	58.3	28.3	32.6

Table 2. The total acids, amino nitrogen and soluble carbohydrate content in 100 grams of white glutinous rice fermented with ragi tape R.

Incubation time (hours)	Moisture (gr)	Total Solids (gr)	pH	Total Acids (mg)	Amino Nitrogen (mg)	Soluble carbohydrate as glucose (mg)
0	54.21	45.79	6.17	163	–	65.91
24	55.00	45.00	4.56	557	–	95.50
48	60.33	39.67	3.34	1756	37.2	120.68
96	61.03	38.97	3.86	3067	91.8	116.68
120	63.88	36.12	3.75	2467	79.3	92.03

pressed to separate the juice from the substrate. The results are presented in Table 1.

The highest juice production was obtained on the fifth day using an inoculum concentration of 0.375% (w/w). These results were in accordance with the traditionally way of preparation of Brem Bali. People use one ragi cake for 1 kg of glutinous rice to obtain one litre of tape juice.

The weight of one such ragi cake is 3.75 grams. The tape juice from white glutinous rice had a very light yellow colour. It turned darker after some time and if daily exposed to daylight the colour will become dark brown like concentrated tea. When black glutinous rice was used, the tape juice had the colour of red wine, later turned purple – black.

The residue of the tape made with the inoculum ragi tape R was rather dry, while the one with ragi tape S was sticky and wet due to the high sugar content.

Analysis of the changes of soluble material occurring in the substrate during the fermentation of white glutinous rice were carried out with ragi tape R only (0.375%, w/w). The results are presented in Table 2.

During fermentation an increase in moisture and a decrease in total solids occurred as a result of the activities of the microorganisms. The total acids and amino nitrogen increased until 96 hours of incubation, then decreased. The decrease might be due to reactions that occurred between the various compounds in the substrate. The pH decrease indicated the production of acid.

The alcohol content during the fermentation process was also examined. The results are presented in Table 3.

After three days of the fermentation at room temperature (30°C) when the tape could still be consumed as a snack, the alcohol content was about approximately 7% for both inocula used. On the fifth day the alcohol content was 15.2% and 14.6% respectively for inocula R and S. While on the sixth day there was still a slight increase. However, the tape obtained had developed an unpleasant taste which made the product not consumable

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Table 3. The alcohol content of Brem Bali made respectively with inoculum ragi tape R and S.

	Time (days)	Alcohol content (%) Inoculum R	Inoculum S
Juice	3	7.50	7.82
	4	8.70	9.00
	5	15.20	14.60
	6	16.60	16.60
Juice	30	18.15	17.15
	90	17.48	16.43
	120	14.80	14.80
	150	14.50	14.40

anymore. As seen in the table, the juice which was stored at room temperature for 30 days showed an increase in alcohol content and then a decrease when kept for longer period. A few yeast cells were found still alive. This decrease might be due to reactions between the alcohol and organic acids produced to form esters or others which contributed to the taste, aroma and colour. The longer the tape juice was stored, the darker the colour became (Pearson, 1970).

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Lodder, J. (ed.) 1971. The Yeast. A taxonomic Study. North Holland Publishing Company. Amsterdam.

Pearson, D. 1970. The Chemical Analysis of Foods. 6th edition. Chem. Publ. C., New York.

Prof John E Smith delivering the Plenary lecture on the role of biotechnology in the economy of developing courtries.

BEST COPY AVAILABLE

Current Research in Biotechnology in India

H Sharat Chandra

Indian Institute of Science, Bangalore 12, India

Abstract

Organized effort in biotechnology is relatively recent in India. I will confine myself to those aspects of biotechnology research which are based on recombinant DNA and on certain aspects of clonal propagation of plants. Barring two or three exceptions such research is largely confined to the universities. Some of the areas under investigation are: i) organization and expression of cytochrome P-450 genes in rat liver; ii) ribosomal proteins in rice embryos; iii) control of gene expression in the silk worm and the cloning of silk fibroin genes; iv) nucleic acid metabolism in nitrogen-fixing bacteria and v) gene expression in the rinderpest virus; vi) colitis page lysozyme and its gene. To facilitate research in these areas of biotechnology, plans are underway to manufacture restriction enzymes, ligases and labelled triphosphates within the country. A National Biotechnology Board has been set up. The Council of Scientific and Industrial Research is setting up a new Institute to focus on research in this and related areas. The Department of Science and Technology, Government of India, has identified research groups in Calcutta, Madurai, Delhi, Aligarh, Bombay, Varanasi and Bangalore to provide training in recombinant DNA technology. All in all, there is a great deal of interest and expectation in the application of the tools of biotechnology for tackling problems in agriculture and health.

Biotechnology of Interferons: The Transition from Natural to Recombinant Human Interferon

Y H Tan

Department of Medical Microbiology & Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Abstract

There has been much discussion about interferon as an anticancer and antiviral agent. How it will be used in the treatment of malignant and viral diseases will become known after the clinical trials are completed. For now, interferon has been shown to be efficacious in the treatment of a number of viral diseases and of two types of tumours suspected of a viral etiology.

Since its discovery about 25 years ago, the study of interferon has been hampered until recently by its scarcity. Interferon is usually not produced by cells until the cells are exposed to an appropriate stimulus – a virus, a double stranded ribonucleic acid or a mitogen. Human white blood cells produce mostly α interferon. Skin and connective tissue cells produce β interferon and T cells produce interferon in response to antigenic stimulus. Once interferon is produced and secreted by a cell, more contact between the other cells and a few molecules of interferon is sufficient to alter the physiologic state of the other cells. In the altered state, the cells will not support viral replication, they become more sensitive to certain chemotherapeutic drugs, cellular DNA synthesis is inhibited and the rate of cell division slows down.

Two principal procedures were developed to produce interferons by human cells. One method established by Kari Cantell was to induce human white blood cells with inactivated Sendai virus. The leukocytes, prepared in large batches from the buffy coats, produced α interferon. Consequently, this method is dependent on the blood transfusion services for buffy coats. I use a different approach, human connective tissue cells were "superinduced" by a specific regime of stimuli to produce β interferon optimally. By both approaches, amounts of α and β interferons have been prepared and are being tested in clinical trials. Both procedures of interferon production have also enabled the production of sufficient amount of human α, β and α interferon messenger RNA leading to the synthesis of their respective genes which are spliced into the DNA of *E. coli* and expressed. This has made possible the commercial scale production of interferon by recombinant bacteria.

Although the recombinant bacteria and human cell produced interferons have identical amino acid sequences there are some differences in their biological activities. The recombinant product in unglycosylated but the human product is. The human product is secreted while the bacterial product is not and sometimes requires a strong denaturating agent to extract it. Interestingly, the differences in biological activities could be eliminated by introducing a site specific change in the DNA sequence of the interferon gene. Such creative biochemical manipulation shows that it is feasible to apply the concept of site-specific mutagenesis to improve a biological product. With the various forms of interferons becoming available, their importance as clinically useful antiviral and antitumour drugs will be established soon.

Recombinant DNA Derived Human Interferons

Budd Colby

IFN Program, Cetus Corporation, Berkeley, CA, USA

Abstract

The interferons (IFN) are inducible proteins of three distinct immunological types IFN-alpha and IFN-beta are produced and excreted by peripherial blood leukocytes and by fibroblasts respectively in response to viral infections. IFN-gamma is produced by mitogen-treated lymphocytes. The IFN's exert a variety of biological properties including the induction of an antiviral state, the inhibition of proliferation of rapidly growing cells, and the modulation of certain functions of the immune system. These activities tend to be species specific and are the result of rather small numbers of IFN molecules. As expected for proteins with high specific activities, cells normally produce preciously small amounts of IFN. Consequently, IFN researchers struggled for some twenty years with inadequate amounts of often impure preparations.

In the past four years the genetic information specifying human IFN's have been introduced into *E. coli* using recombinant DNA technology. The DNA sequences were determined and the amino acid sequences of the corresponding human IFN's were deduced. Further use of this technology has allowed the expression of these human genes in bacteria, yeast and mammalian cells, in some cases with extremely high yields. Recombinant DNA produced human IFN's have been highly purified and their biochemical and biological properties studied in cell culture and animal model systems. Finally, two of the sixteen rIFN-alpha molecules are in Phase III clinical trials as both anticancer and antiviral therapeutics while rIFN-beta and rIFN-gamma have been recently introduced into Phase I trials. The cloning, expression, purification, biological characterization and clinical results of these human interferons will be discussed.

New Sources of Single Pore Cell Protein

Yasuji Minoda

Department of Agricultural Chemistry, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Abstract

Researches for new microbial protein sources of food and feed were focussed on yeast from palm oil and hydrogen bacteria. An isolated strain which was identified with *Torulopsis candida* assimilated effectively crude and refined palm oil. The addition of surfactant, vigorous agitation and high oxygen supply accerelated the yeast cell growth. Optimum temperature for growth was 30°C and optimum pH was 3.0. Cell yield under optimum culture conditions on 3% of palm oil was 95%. The other strain which could grow at around 40°C was isolated and identified with *Candida tropicalis*.

Thermophilic hydrogen bacterium *Pseudomonas hydrogenothermophila* showed very rapid growth (μ = 0.73 hr^{-1}) at 52°C on simple inorganic medium under the supply of mixed gas of hydrogen, oxygen and carbon dioxide. The most suitable medium and the best gas ratio for cell growth were determined. For each kilogram of bacterial cells, 5.4 m^3 of hydrogen was consumed and the carbon dioxide intake ratio was 65%.

Protein contents of *T. candida and P. hydrogenothermophila* were 46.8 and 78.0% respectively and the amino acid compositions of both proteins were good balanced. These organisms were thought to be promising strains for single cell protein production.

CLOSING SESSION OF SYMPOSIUM

Summary

This symposium has demonstrated very clearly that biotechnology encompasses a very wide diversity of techniques and applications. It is apparent that developing countries are also widely diverse and the role and application of biotechnology cannot be generalized across all developing countries.

For example, countries such as Singapore and Hong Kong lack natural physical resources, but they are rich in human resources. They have demonstrated their organizing and marketing ability and compete successfully on world markets. They should therefore have the potential to compete successfully at the 'high technology' end of the biotechnology spectrum, for example by the production of human pharmaceuticals new proteins, new varieties of plants and other useful organisms with the necessary genetic changes incorporated. But it is unlikely that even they would have the capacity to cover all of the potential products. Hence it will be necessary to select carefully the most appropriate area and to be aware that for every dollar spent on research, ten dollars will be necessary for development. Such countries should continue to develop their strengths in molecular biology research and development and to stimulate government and business support for development and production.

Other countries, such as Malaysia and Indonesia, have different strengths and different potentials. They are more richly endowed with natural resources. They have well developed agricultural industries such as rubber, palm oil, tea, cocoa etc. They would probably be best served by choosing the appropriate scientific developments with the objective of applying specific aspects of biotechnology to their existing industries. Their needs therefore will be for a core of high grade research directed largely to their local products and the development of small local industries.

India is different again. It has natural resources, well developed agricultural systems and a large local market. Because of this large local market it should be able to follow the Japanese pattern of 'first develop a product for the local market, then extend to world markets'. In this respect India should be capable of developing products and eventually capturing a share of the world market. Because it has well developed agricultural systems and a large rural population it should also look to the application of biotechnology to the further development of local industries.

It becomes too lengthy to elaborate the special characteristics of all of the developing countries represented at this symposium, but the participants from all countries were urged to combine their knowledge of biotechnology with the special conditions existing in their own countries and to promote those aspects most likely to be successful.

A. N. Rao
R. D. Brock

SYMPOSIUM ON
RESEARCH IN BIOLOGY AND BIOTECHNOLOGY IN DEVELOPING COUNTRIES
SINGAPORE, 2–4 NOVEMBER, 1983

PROGRAMME

Wednesday, 2 November 1983

REGISTRATION AND OPENING CEREMONY

0830 – 0930	Registration of Participants, BioScience Complex, National University of Singapore
0930 – 0945	Welcome Address: Dr Chua Sian Eng President, Singapore Institute of Biology
0945 – 1000	Welcome Address: Professor S. Radhakrishna Scientific Secretary, COSTED
1000 – 1015	Opening Address: Dr Tay Eng Soon Minister of State for Education
1015 – 1045	Reception/Exhibition of Scientific Equipment

Friday, 4 November 1983

CLOSING SESSION
Conference Room 1

1700 – 1730	Closing Remarks
1730	Reception hosted by Singapore Institute of Biology

PLENARY

Wednesday, 2 November 1983

PLENARY SESSION I
Chairman: Professor A.N. Rao
Conference Room 1

1045 – 1130	Role of Biotechnology in the economy of developing countries – J.E. Smith, University of Strathclyde, Glasgow, UK
1130 – 1215	Discussion
1215 – 1330	Lunch

PLENARY SESSION II
Chairman: Assoc Professor T.J. Lam
Conference Room 1

1330 – 1350	Current research in Biotechnology in India – H. Sharat Chandra, Indian Institute of Science, India
1350 – 1410	The progress of Biotechnological research in Thailand – Nalin Nilubol, Chulalongkorn University, Thailand
1410 – 1430	Biotechnology methods useful for improving the palm oil in Malaysia – S.C. Cheah, Palm Oil Research Institute of Malaysia, Malaysia
1430 – 1450	Biotechnology research under progress in Indonesia – Suhirman, National Biological Institute, Indonesia
1450 – 1510	Biotechnology research in Singapore – A.N. Rao, National University of Singapore, Singapore
1510 – 1530	Discussion
1530 – 1600	Coffee/Tea Break/Exhibition

PLENARY SESSION III
Chairman: Dr W.H. Tan
Conference Room 1

1600 – 1635 Biotechnology of interferons: The transmission from natural to recombinant human interferon
– Y.H. Tan, University of Calgary, Alberta, Canada

1635 – 1710 Recombinant DNA derived human interferons
– C.B. Colby, Cetus Corporation, California, U.S.A.

1710 – 1730 Discussion

BIOTECHNOLOGY

Thursday, 3 November 1983

BIOTECHNOLOGY I
Chairman: Professor G. Lim
Conference Room 1

0845 – 0920 Gene transfer and somaclonal variation for plant improvement
– R.D. Brock, CSIRO, Australia

0920 – 0955 New sources of single cell protein
– Y. Minoda, University of Tokyo, Japan

0955 – 1015 Discussion

1015 – 1045 Coffee/Tea Break

1045 – 1700 **CONCURRENT SCIENTIFIC SESSIONS**

APPLIED BIOLOGY
TOXICOLOGY & PEST MANAGEMENT
ECOLOGY
BIOCHEMISTRY, PHYSIOLOGY & GENETICS

Friday, 4 November 1983

BIOTECHNOLOGY II
Chairman: Professor R. Hori
Conference Room 1

0845 – 0915 Recent advances in Biotechnology with special reference to developing countries
– K.F. Gregory, University of Guelph, Canada

0915 – 0945 Biotechnology – Manpower needs
– S. Krishnaswamy, Madurai Kamaraj University, India

0945 – 1015 Discussion

1015 – 1045 Coffee/Tea Break

1045 – 1700 **CONCURRENT SCIENTIFIC SESSIONS**

APPLIED BIOLOGY
TOXICOLOGY & PEST MANAGEMENT
ECOLOGY
AQUACULTURE
BIOCHEMISTRY, PHYSIOLOGY & GENETICS

APPLIED BIOLOGY

Thursday, 3 November 1983

APPLIED BIOLOGY I
Chairman: Dr K.H. Loke
Conference Room 1

1045 – 1100 Regulation of the human beta interferon gene
– Lee, K.L. & Y.H. Tan, University of Calgary, Alberta, Canada

1100 – 1115 An ELISA for beta human interferon
– Inoue, M, E. Konno, C.B. Colby & Y.H., Tan University of Calgary, Alberta, Canada

1115 – 1130 The application of recombinant DNA techniques for the study of human genetic diseases
– Tan, J.W.O., K.C. Tan-Un, Y.K. Woo & V.S.M. Lam, University of Hong Kong, Hong Kong

1130 – 1145 Application of histochemical dyes as functional groups in chromatography
– Madaras, F. & M.Y. Chew, CM Chemicals, Australia

1145 – 1200 Protein binding specificity of Bromocresol green
– Chew, M.Y. & F. Madaras, CM Chemicals, Australia

1200 – 1215 Labelling fishes with an activable element
– Prof. Reji Hori, National University of Singapore, Singapore

APPLIED BIOLOGY II
Chairman: Mr M. Nadchatram
Conference Room 1

1330 – 1345 Phytotoxicity of refuse compost during the process of maturation
– Wong, M.H., Y.P. Cheung & L.M. Chu, Chinese University of Hong Kong, Hong Kong

1345 – 1400 Immobilisation of amyloglucosidase
– Sastry, C.A. & V. Tatkar, Centre for Biosciences and Biotechnology, IIT, Madras, India

1400 – 1415 Studies on characteristics and treatment of waste water from an industry manufacturing sodium alginate – A case study
– Rao, G.B.V.S. & C.A. Sastry, Department of Chemical Engineering, IIT, Madras, India

1415 – 1430 Biomass utilisation for bio-gas production
– Rao, M.N., S.J. Polytechnic Campus, Bangalore, India

1430 – 1445 Management of aquatic weeds in fish culture as a means of waste utilization
– Ramaprabhu, T., Central Inland Fisheries Research Substation, Cuttack, India

1500 – 1530 Tea/Coffee Break

APPLIED BIOLOGY III
Chairman: Dr Y.K. Lee
Conference Room 1

1530 – 1545 Biodeterioration of vegetable seeds
– Raghavan, L. & G. Lim, National University of Singapore, Singapore

1545 – 1600 **Schizochytrium** sp. associated with external lesions of some estuarine fishes
– Loganathan, B. et al., Annamalai University, India & National Institute of Oceanography, India

1600 – 1615 Brem Bali fermentation
– Gandjar, I., D.S. Slamet, I.R. Koentjoro, University of Indonesia, National Research & Development Center, Indonesia

1615 – 1630 Experimental field cultivation of **Gracilaria edulis**
– Sivapalan, A. & Theivendirarajah, University of Jaffna, Sri Lanka

1630 – 1645 Food filtering process of **Daphnia magna** strauss to assess the water soluble detergent
– Sastrodihardjo, S. & H. Zulkifir, Institut Technology Bandung, Indonesia

Friday, 4 November 1983

APPLIED BIOLOGY IV
Chairman: Assoc Professor C.S. Hew
Conference Room 1

1045 – 1100 Axenic mixed population culture. A new approach in photosynthetic algal cultivation for production of biomass
– Lee, Y.K., National University of Singapore, Singapore

1100 – 1115 The use of animal tissue culture in virus studies
– Ng, M.L., National University of Singapore, Singapore

1115 – 1130 Cleansing of bivalve shellfish in Hong Kong using the ultra violet depuration system
– Kueh, C.S.W., Hong Kong Polytechnic, Hong Kong

1130 – 1145 Utilisation and management of agricultural wastes in the Philippines
– Uyenco, F.R., University of Philippines, Diliman, Philippines

1145 – 1200 Commercial production of the Japanese Quail, **Corturnix corturnix japonica**, in Singapore
– Teoh, K.W. et. al., National University of Singapore, Singapore

1200 – 1215 Interfacing microcomputer to experiment
– Lua, K.T., National University of Singapore, Singapore

TOXICOLOGY AND PEST MANAGEMENT

Thursday, 3 November 1983

TOXICOLOGY AND PEST MANAGEMENT I
Chairman: Assoc Professor K.L. Chan
Conference Room 2

1045 – 1100 Effects of prey quantity and type on **Cyrtorhinus lividipennis** (Reuter) (Miridae: Hemiptera), a predator of rice brown plant hopper
– Chua, T.H. & E. Mikil, Universiti Malaya, Malaysia

1100 – 1115 The introduction of exotic parasitoids to control **Plutella xylostella** (L.) in Malaysia
– Ooi, A.C. Peter & G.S. Lim, MARDI, Malaysia

1115 – 1130 Ovicidal action of Deltamethrin on **Plutella xylostella L.**
– Ho, S.H. & P.M. Goh, National University of Singapore, Singapore

1130 – 1145 Toxicological studies of esterases of susceptible and insecticide-resistant **Plutella xylostella L.**
– Sudderuddin, K.I. & A. Marappan, Singapore

1145 – 1200 Effect of DDT and Methyl parathion on the acetylcholinesterase activity of air breathing fish **Channa striatus**
– Bhaskaran, R. & T.J. Pandian, APA College & Madurai Kamaraj University, India

1215 – 1330 Lunch

1500 – 1530 Tea/Coffee Break

TOXICOLOGY AND PEST MANAGEMENT II
Chairman: Mr M. Nadchatram
Conference Room 2

1530 – 1545 Changes in water content and ionic regulation of the bullfrog, **Rana tigrina** due to two insecticides
– Deshmukh, P.B. & R. Keshavan, Science College, India

1545 – 1600 Efficacy of chitin synthesis inhibitors in controlling Hemipteran insect pests
– Chockalingam, S. et. al., Thiagarajar College, India

1600 – 1615 Use of chitin synthesis inhibitors in the management of mosquito
– Chockalingam, S. et. al., Thiagarajar College, India

Friday, 4 November 1983

TOXICOLOGY AND PEST MANAGEMENT III
Chairman: Dr V. Phang
Conference Room 2

1530 – 1545 Organochlorine residues in bats in Thailand
– Kruthanut, S., Thailand Institute of Scientific and Technological Research, Thailand

1545 – 1600 Residues of organochlorine pesticides in the sediments of vellar estuary, S. India
– Rajendran, N. & V.K. Venugopalan, Center of Advanced Study in Marine Biology, India

1600 – 1615 Delayed neurotoxicity and inhibition of protein synthesis in parabromophenylacetylurea and methyl-mercury poisoning in the rat
– Chen, F.C., National University of Singapore, Singapore

1630 – 1646

1645 – 1700

ECOLOGY

Thursday, 3 November 1983

ECOLOGY I
Chairman: Dr Y.C. Wee
Conference Room 3

1045 – 1100 Fungi in the freshwaters of Singapore
– Tan, T.K. & G. Lim, National University of Singapore, Singapore

1100 – 1115 Simulating resource utilisation with age-structured arrays under logistic-type regulation
– Murphy, D.H., National University of Singapore, Singapore

1115 – 1130 Enteric luminous bacteria of bivalves from Porto Novo waters
– Ramesh, A. et. al., Annamalai University, India

1130 – 1145 Biomass production and leaf litter production of **Pandanus** spp. in the Tasek Bera swamp forest, Malaysia
– Verghese, S. & J.L. Furtado, National University of Singapore, Singapore & Commonwealth Secretariat, U.K.

1145 – 1200 Litter fall in a hill dipterocarp forest in relation to climatic data
– Fong, F.W. & S.M. Ng, University of Malaya, Malaysia

1200 – 1215 A computer simulation study of the Gower's general coefficient of similarity
– Lim, T.M. & H.W. Khoo, National University of Singapore, Singapore

1215 – 1330 Lunch

ECOLOGY II
Chairman: Dr Y.C. Wee
Conference Room 3

1330 – 1345 Ecological distribution of reef organisms at Pulau Salu
– Chou, L.M. & F.J. Wong, National University of Singapore, Singapore

1345 – 1400 Studies on the bionomics of interstitial fauna of coast of Southeast India
– Ayyakkannu, K., Annamalai University, India

1400 – 1415 Ecological studies for conservation of birds in Songkhla Lake
– Porntip Angkapreechases, Thailand Institute of Scientific & Technological Research, Thailand

1415 – 1430 Reproductive constraints in tropical aerial feeding birds
– Hails, C.J., Botanic Gardens, Singapore

1430 – 1445 Habitat preferences in branchiopod fauna of lower deltaic region of River Krishna, South India
– Radhakrishna, Y. & M.K.D. Prasad, Nagarjuna University, India

1445 – 1500 Bat pollination in Thailand
– Pakarnseree, L., Thailand Institute of Scientific & Technological Research, Thailand

1500 – 1530 Tea/Coffee Break

Friday, 4 November 1983

ECOLOGY III
Chairman: Dr R. Corlett
Conference Room 2

1045 – 1100 Contamination of roadside soil and vegetation with Pb, Cu and Zn as a function of distance from the road in Hong Kong
– Ho, Y.B. & K.M. Tai, University of Hong Kong, Hong Kong

1100 – 1115 Plants and particulate pollutants of the air
– Das, T.M., Calcutta University, India

1115 – 1130 Effect of temperature on the energy balance of **Mantis religiosa** (Dictyoptera: Mantidae)
– Muthukrishnan, J., APA College, India

1130 – 1145 The shell peeling structure on the chelipeds of the Box crab, **Calappa philargius** Linn., and other crabs in relation to mollusc architecture
– Ng, K.L.P. and L.W.H. Tan, National University of Singapore, Singapore

1145 – 1200 Significance of habitat features on the distribution of Indian Caecilians (Apoda: Amphibia)
– Balakrishna, T.A., Vijaya College, Bangalore, India

1215 – 1330 Lunch

1500 – 1530 Tea/Coffee Break

ECOLOGY IV
Chairman: Dr K.H. Chow
Conference Room 3

1530 – 1545 Specific dynamic action in **Lates calcarifer**
– Nandakumar, R. et. al., Annamalai University, India

1545 – 1600 Reproductive energetics of the anabantid **Colisa lalia**
– Hails, A.J., University of Malaya, Malaysia

BIOCHEMISTRY, PHYSIOLOGY & GENETICS II
Chairman: Dr C.L. Poh
Conference Room 4

1330 – 1345 Influence of hypercholesterolemia and onion extract on the structure and functioning of erythrocytes
– Singh, M. & P. Kanakaraj, IIT, Madras, India

1345 – 1400 Role of hormones in larval growth and development in fishes
– Lam, T.J. National University of Singapore, Singapore

1400 – 1415 Endocrine control of reproduction in the prawn, **Caridina weberi**
– Nagabushanam, R. & T.S.N. Reddy, Marathwada University, India

1415 – 1430 Hormonal control of ovarian activity in the water leach, **Poecilobdella viridis** (Blanchard)
– Kulkarni, G.K. **et. al.**, Marathwada University, India

1500 – 1530 Coffee/Tea Break

Friday, 4 November 1983

BIOCHEMISTRY, PHYSIOLOGY & GENETICS III
Chairman: Dr C. Epp
Conference Room 2

1045 – 1100 Biochemical changes during reproductive cycle in the prawn, **Macrobrachium lamerrii**
– Sarojini, R. **et. al.**, Marathwada University, India

1100 – 1115 Biochemical variations in the muscles and hepatopancreas in relation to sex and size in the crab **Merippe rumphii** (Fabr.)
– Babu, E.D. & K. Shyamasundari, Andhra University, India

1115 – 1130 Hatching and larval development of **Mesocyclops leucarti** (Cyclopoidea: Copepoda)
– Gita Paul, Fatima College, India

1130 – 1145 Effects of photoperiod on the growth and survival of **Pseudemys scripta elegans** hatchlings
– Chou, L.M. & P. Venugopal, National University of Singapore, Singapore

1145 – 1200 Biochemical fluctuation during ovarian development in the edible crab **Scylla serrata** (Forskal)
– Ezhilarasi, S. & T. Subramonian, University of Madras, India

1215 – 1330 Lunch

BIOCHEMISTRY, PHYSIOLOGY & GENETICS IV
Chairman: Dr K.K. Ho
Conference Room 4

1045 – 1100 Serological studies on the egg maturation in the edible crab **Scylla serrata** and their application to aquaculture
– Ezhilarasi, S. & T. Subramonian, University of Madras, India

1100 – 1115 The role of accessory reproductive glands' secretion in the maturation of spermatozoa and spermatophore formation in the scorpion, **Heterometrus fulvipes**
– Mahalingam, S. & T. Subramonian, University of Madras, India

1115 – 1130 Physiological energetics of food conversion in a few culturable freshwater catfishes of India
– Reddy, R.S., Bangalore University, India

1130 – 1145 Expression of sex-limited colour pattern genes in selected varieties of female guppies (**Poecilia reticulata**) with androgen treatment
– Fernando, A.A. & V.P.E. Phang, National University of Singapore, Singapore

1200 – 1215 A continuous cell line from male-looking gravid toad, **Bufo melanostrictus**, and metaphase chromatin
– Sit, K.H., National University of Singapore, Singapore

1215 – 1230 Faecal weight as an index of development rate and calorific content of imago of Lepidopterous insects
– Muthukrishnan, J. & T.J. Pandian, M.K. University, India

1230 – 1330 Lunch

BIOCHEMISTRY, PHYSIOLOGY & GENETICS V
Chairman: Mr F. Madaras
Conference Room 2

1330 – 1345 Bovine factor V and its effect on prothrombin activation
– Ong, T.C., National University of Singapore, Singapore

1345 – 1400 Langur monkey as a model for research in reproduction
– Lohiya, N.K. et. al., AILMS, University of Delhi, India

1400 – 1415 Congenital myopathic ducks – animal model for human disease
– Gopalakrishnakone, P. & F.C.T. Voon, National University of Singapore, Singapore

1415 – 1430 Evaluation of Krebs-Ringer-Tris maleate buffer as an incubation medium for **Hymenolepis diminuta**
– Ip, Y.K., National University of Singapore, Singapore

1500 – 1530 Tea/Coffee Break

BIOCHEMISTRY, PHYSIOLOGY & GENETICS VI
Chairman: Dr M.L. Ng
Conference Room 4

1530 – 1545 Effect of deleting inositol from the host diet on the establishment and growth of **Hymenolepis diminuta** (Cestoda)
– Ip, Y.K., National University of Singapore, Singapore

1545 – 1600 Molecular nitrogen fixation among strains of **Tolypothrix**
– Kamalaveni, R. & S. Shanmugasundaram, Madurai Kamaraj University, India

1600 – 1615 Control of oviposition by prostaglandins and indomethacin in chickens
– Chen, T.W. et. al., National University of Singapore, Singapore

1615 – 1700 Cine-photomicrographic study of an isolated cell.
1. A complementary phase in somatic division.
2. Transport of micromolecules through living membrane.
3. Displacement of nucleus by physical force.
4. Absorption of particular pollutants by the leaf-hair cell.
– Das, T.M. et. al., Calcutta University, India

Prof S Radhakrishna, Secretary, COSTED, addressing the gathering.

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We are very thankful to Miss Joan Choo, Mdm Chan Siew Khim, Mrs Jessie Mui and Miss Doreen Tan who helped us in many ways both in organising the Symposium and in the editorial matters.

A. N. RAO
Editor

SYMPOSIUM ON
RESEARCH IN BIOLOGY AND BIOTECHNOLOGY
IN DEVELOPING COUNTRIES
2 – 4 NOVEMBER, 1983

Dr Khoo Hong Woo, Chairman, Organising Committee, addressing the gathering.

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SYMPOSIUM ON RESEARCH IN BIOLOGY & BIOTECHNOLOGY IN DEVELOPING COUNTRIES

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